The role of multiple host species in shaping the transmission dynamics of *Bartonella* parasites within natural rodent communities.

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Summary

Diseases caused by parasites are responsible for immense human and animal suffering, declines in biodiversity, and substantial economic losses across the globe. It is therefore important to understand how parasites spread through and persist within natural populations, so that control interventions that aim to reduce individual infection risk can be designed and implemented appropriately. Crucially, most parasites exist within multi-host communities, and often appear to infect multiple sympatric species, all of which potentially play a role in parasite persistence. However, certain species may contribute disproportionately to transmission and be nearly completely responsible for the persistence of a parasite within a community. Identifying such "key hosts" therefore offers a means to appropriately target control interventions to maximise success. However, assessing host species contributions to parasite transmission within multi-host communities is a challenging task, and much insight can be gained from studies of model host-parasite systems. In this thesis, the transmission dynamics responsible for the persistence of several endemic *Bartonella* parasites (bacterial flea-borne haemoparasites) are investigated within wild sympatric populations of wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*) in northwest England. *Bartonella* infections were first identified to species-level according to an existing method based on length polymorphism of a fragment of the 16S-23S internal transcribed spacer region (ITS). Broad patterns of prevalence suggested that some species were host generalists while others were host-exclusive, indicating that different transmission dynamics underlie the persistence of each *Bartonella* species within the rodent community. Attempts to identify key transmission hosts for each *Bartonella* species based on the effect of past host population densities on infection risk proved inconclusive. However, finer-scale characterisation of *Bartonella* infections, using DNA sequencing, found that *Bartonella* species that appear to be generalists actually comprise a complex of genetic variants, the majority of which are host-specific, suggesting that transmission between host species is uncommon and limited to a relatively few host-shared variants. Furthermore, detailed characterisation of the flea community infecting wood mice and bank voles found that these *Bartonella* vectors were host-generalists, and that at least two flea species were able to transfer between individuals of different host species. This suggests that a lack of between-species transmission is likely to arise through different compatibility between host species and *Bartonella* variants, rather than as a result of current ecological encounter barriers (e.g. through differential *Bartonella*-flea or flea-rodent specificity). The results of an experimental manipulation of between-species transmission within these wild communities support the notion that between-species transmission of *Bartonella* parasites is uncommon. Across three woodland sites, bank voles were treated with a veterinary insecticide to remove their fleas and therefore reduce the rate of transmission of *Bartonella* from treated bank voles to the rest of the rodent community. Following treatment, risk of bank vole infection with bank vole-exclusive *Bartonella* variants was reduced, but there was no affect on the risk of bank vole infection with host-shared variants, nor risk of infection in wood mice with either wood mouse-exclusive or host-shared variants. Importantly, these treatment effects were best identified by grouping the parasites on a 'functional' (i.e. host-exclusive versus host-shared variants) rather than a taxonomic (i.e. *Bartonella* species) basis. Together, these findings highlight the importance of characterising parasite infections to as fine a scale as possible, and the value of using a combination of observation, genetic and experimental approaches to understand parasite transmission within complex natural multi-host systems.

Chapter 1

General introduction

1.1 Motivation for studying parasite transmission in multi-host communities

Parasites, including microparasites (e.g. viruses, bacteria, protozoa) and macroparasites (e.g. helminths, ectoparasites), are ubiquitous in nature, and are increasingly recognised as important components of natural communities (Marcogliese, 2004; Lafferty *et al*., 2006; Wood *et al*., 2007; Tompkins *et al*., 2011). Worldwide, diseases caused by parasitic infections are responsible for immense human and animal suffering, declines in biodiversity, and substantial economic losses (Daszak *et al*., 2000; Morens *et al*., 2004). Furthermore, emerging infectious diseases (EIDs) represent an on-going threat to human health, with a high proportion believed to have a zoonotic origin (Taylor *et al*., 2001; Jones *et al*., 2008). It is therefore important to understand how parasites spread through and persist within natural populations, so that control interventions that aim to reduce individual infection risk can be designed and implemented appropriately.

Of crucial importance to the successful design of disease control interventions is the fact that most parasites can infect more than one host species, and often appear to infect multiple sympatric species within natural communities (Cleaveland *et al*., 2001; Woolhouse *et al*. 2001; Pedersen *et al*., 2005; Begon *et al*., 1999). The simplistic 'single host species – single parasite species' paradigm dominated early studies of host-parasite ecology, and while many important advances have been made under this framework (e.g. Anderson & May 1979) the need to address the community context of host-parasite ecology is being increasingly recognised (Taylor *et al*., 2001; Woolhouse *et al*., 2001; Jones *et al*., 2008). One reason for this is that transmission between host species is likely to influence the force of infection within the community and may be necessary for parasite persistence within different host species; therefore an individual's risk of infection may be determined, in part, by the structure of the host community and the nature of parasite transmission within it (i.e. the direction and relative rates of

transmission within and between host species; Bowers & Begon, 1991; Holt *et al*., 2003; Dobson, 2004; Fenton & Pedersen, 2005).

The nature of parasite transmission, dynamics and persistence is likely to be complex in most multi-host communities. This is because host species within a community are unlikely to contribute equally to parasite transmission, due to differences in abundance or underlying heterogeneities related to exposure, susceptibility and immunity, between the potential host species (Haydon *et al*., 2002; Altizer *et al*., 2003; Kilpatrick *et al*., 2006; Streicker *et al*., 2013). Certain species may contribute disproportionately to transmission and be nearly completely responsible for the persistence of a parasite within a community and the infection rates of other sympatric host species (Streicker *et al*., 2013). Identifying such "key hosts" therefore offers a means to appropriately target control interventions to maximise success (e.g. Rudge *et al*., 2013; Donnelly et al. 2006; Kaare et al. 2009). Conversely, several host species may each contribute substantially to persistence and abundance of a given parasite, suggesting an alternative control strategy may be preferable. However, assessing host species contributions to parasite transmission and identifying whether there is a key host species within multi-host communities is a challenging task.

1.2 Studies of parasite transmission in multi-host communities

1.2.1 Conceptual frameworks of parasite transmission in multi-host communities

Several important theoretical and conceptual frameworks have been developed that seek to describe interactions between parasites and hosts within multi-host communities. There is a growing appreciation that parasitic infections play a role in regulating host population dynamics (e.g. Hudson & Greenman, 1998; Tompkins *et al*., 2003), and dynamic mathematical models have been used in several studies to describe how a shared parasite may affect host population dynamics and co-existence within multi-host systems (Holt & Pickering, 1985; Bowers & Begon, 1991; Begon & Bowers, 1994; Greenman & Hudson, 2000). Other approaches have focused on the parasite's

perspective, and have sought to identify how multiple host species within a community affect parasite dynamics and persistence (e.g. Dobson, 2004). Following this latter type of approach, Haydon *et al*. (2002) defined host populations in terms of their ability to sustain parasite transmission in the absence of transmission from another source, based on whether population size exceeded a critical threshold (i.e. critical community size). Populations or assemblages of host species that exceed this threshold are termed "maintenance" populations or communities, and are key for parasite persistence within the community. Importantly, the authors also highlighted the significance of other types of host species that do not necessarily constitute maintenance populations in their own right, but may nevertheless be crucial for parasite persistence within a "target" host species (i.e. the host species of particular interest), therefore expanding earlier, simplistic views of reservoirs as non-pathogenic, single species populations. For example, host populations that transmit infection directly to a target population are termed "source" populations. Such host populations may not be important for parasite persistence in the community in general, but blocking transmission from the source to the target host population may be an efficient means of control for the target population.

Holt *et al*. (2003) expanded the above concept by developing a graphical isocline framework to describe how the nature of transmission between host species (i.e. the relative rates of intra- and inter-species transmission) affects the combination of host densities required for parasite persistence within a theoretical two-host community. For example, among six different scenarios, they found that if transmission between host species never occurs, then parasite persistence within the community requires the density of at least one of the host species to be greater than the critical threshold for persistence (i.e. when the basic reproductive rate of the parasite is >0). In another scenario, where between-species transmission is more common than transmission between conspecifics, then persistence will depend on whether the densities of both host species combined exceeds the critical threshold, and that persistence is more likely (i.e. $R_0 > 1$) if a mixture of each host species is present.

The broad concept of disease persistence within multi-host communities as a function of host densities, proposed by Haydon *et al*. (2002) and Holt *et al*. (2003), provides a clear practical means by which to identify appropriate strategies for control interventions. However, using critical density thresholds to identify the assemblages of host species

that are important for parasite persistence subsumes the dynamic processes of within- and between-species transmission that determine those critical threshold densities. For example, transmission rate between two host species may depend on the rate of contact between individuals of each species, which may be independent of population size. Other frameworks have developed this idea and sought to explicitly describe how differences in the relative rates of within- and between-species transmission might affect parasite establishment and persistence in the community as a whole, or in particular constituent host species populations. For example, the framework developed by Fenton & Pedersen (2005) describes how different relative rates of net intra- and inter-species transmission will impact on the likelihood of parasite emergence into novel host species, and whether or not it can persist in populations of this novel host in the absence of regular transmission from elsewhere (see also Viana *et al*., 2014). Crucially, they describe how ecological and evolutionary characteristics of hosts and pathogens (e.g. how often host species encounter one another, and the propensity of pathogens to evolve to exploit new host species) are likely to affect the net relative rates of intra- and inter-species transmission and therefore the underlying mechanisms involved in parasite persistence within the host community.

Importantly, these theoretical studies have highlighted the fact that similar patterns of parasite prevalence within host populations may result from very different underlying transmission processes (Fenton & Pedersen, 2005; Viana *et al*., 2014). At one extreme, a parasite common to two host species may only be able to persist within one species due to regular spillover transmission (Antonovics *et al*., 2002; Power & Mitchell, 2004) from the other maintenance host species (i.e. "apparent multi-host parasite"; Fenton & Pedersen, 2005). For example, rabies virus infections in wild carnivores in the Serengeti are only maintained through regular transmission from domestic dogs (Lembo *et al*., 2007). At the other extreme, regular transmission may only occur between conspecifics of each host species, and this within-species transmission may be enough to allow independent disease persistence in both species, even in the absence of between-species transmission (i.e. "true multi-host parasite", maintained by either species alone; Fenton & Pedersen, 2005). For example, brucellosis infections in Yellowstone National Park are maintained endemically within populations of cattle, bison and elk (Dobson & Meagher, 1996). Importantly, these different dynamics of transmission within and between species could lead to similar patterns of prevalence in the two host populations,

but for very different reasons, and must be differentiated in order for effective control programmes to be designed.

1.2.2 Empirical studies of parasite transmission in multi-host communities

Conceptual frameworks have provided important insight into the potential for host species to contribute disproportionately to parasite persistence within multi-host communities, and how this may affect the success of disease control programmes. However, there is a relative paucity of empirical studies that examine the community context of host-parasite interactions within natural populations.

Where empirical studies have been undertaken, they have often revealed complex, and sometimes counter-intuitive, patterns of parasite transmission within natural communities. For example, in the USA, West Nile Virus (WNV) is an emerging zoonotic pathogen, transmitted by mosquitoes, usually between a variety of bird species, but also occasionally infecting mammals, including humans (Kramer & Bernard, 2001). Contrary to earlier hypotheses that persistence within avian communities was driven by the abundant and wide-spread House Sparrow (*Passer domesticus*; Komar *et al*., 2001), a study of mosquito feeding patterns in urban areas of northeastern USA found that the majority of WNV-infectious mosquitoes arose as a result of feeding on a less abundant bird species, the American Robin (*Turdus migratorious*; Kilpatrick *et al*., 2006). Thus, control of WNV-epidemics is likely to require focused efforts targeting this less common species.

The transmission dynamics of *Borrelia burgdoferi*, the causative agent of Lyme disease, has also attracted much attention, and studies of this system in the USA have evoked widespread discussion over the general role of biodiversity in determining disease risk, especially in relation to vector-borne diseases (LoGuiduce *et al*., 2003; Keesing *et al*., 2010; Randolph & Dobson, 2012; Wood & Lafferty, 2013). This bacterium is transmitted by immature stages of *Ixodes scapularis* ticks, which is a generalist ectoparasite of a wide range of mammals, birds and reptiles. However, only some of

these host species are competent hosts for *B. burgdoferi*, including the white-footed mouse (*Peromyscus leucopus*), which is common in degraded (low biodiversity) as well as pristine (high biodiversity) habitats. This has led to suggestions that increased host community diversity may "dilute" the risk of zoonotic Lyme disease by redistributing the bites of the vector population across a range of less competent host species (LoGuidice *et al*., 2003). However, others have highlighted the possibility that increased biodiversity may "amplify" the risk of vector-borne disease if accompanied by an increase in vector density (e.g. tick-borne louping ill virus; Gilbert *et al*., 2001). Identifying key host species of vector-borne parasites may therefore be compounded by the multidimensional impacts of host species on infection risk, through their effects on vector abundance and parasite prevalence.

1.3 Methods for investigating between-species parasite transmission in multi-host communities.

1.3.1 Molecular characterisation of parasite communities

Ultimately, both theoretical and empirical studies to date have suggested that observational approaches, that seek to determine the role of multiple host species in parasite persistence within natural communities, are limited in the information they can provide. Hence, a clear understanding of the nature of transmission within multi-host communities is likely to only be achieved by an integration of several methodologies (Viana *et al*., 2014). Genetic characterisation of parasites is one such approach that is increasingly recognised as a useful tool for delineating transmission routes.

Recognition of the relatively rapid rate of parasite evolution, especially so in viruses due to their high mutation rate, has led to the development of sophisticated analytical approaches for tracing the routes of parasite transmission between individuals and host species. Such approaches are based on comparing the genetic relatedness of pathogens isolated from infected individuals. For example, lineages of rabies virus in North American bats show strong host species associations, such that relatively rare transmission events that occur between species (including from bats to humans) are

identifiable. In light of this, Streicker *et al*. (2010) found that the rate of between-species transmission events between pairs of bat species increased with the phylogenetic relatedness of bat species, and was not explained by the amount of interspecies contact. In the UK, whole genome sequencing of *Mycobacterium bovis* (the causative agent of bovine tuberculosis) found a high degree of similarity between the bacteria infecting cattle and nearby badger populations (Biek *et al*., 2012), indicating on-going interactions between these two host species, although the direction of transmission could not be elucidated.

It is not always feasible to collect genetic information from the parasites infecting enough individuals in a community to achieve the resolution necessary to elucidate chains of transmission at the individual level. However, determining the degree of relatedness between populations of a parasite species infecting populations of different sympatric host species may still reveal useful information regarding parasite transmission dynamics at the community level. The genetic diversity of parasite populations is often high (Poulin & Keeney, 2008), therefore identifying how this diversity is distributed across host species may indicate the rate or net direction of between-species transmission. For example, Wang *et al*. (2006) characterised the population structure of the miracidia (transmission stages) of the parasitic trematode *Schistosoma japonicum* (the causative agent of schistosomiasis) expelled by seven different host species in China, and found that parasites infecting humans and bovids were more similar to each other than either were to parasites infecting goats, pigs, dogs or cats. This suggested that transmission between humans and bovines was more common than between humans and other domestic animals. Indeed recent theoretical work has demonstrated that transmission of *S. japonica* in marshy areas of this study region is driven by bovine species, which act as the maintenance host, and so local elimination of transmission, and therefore infection risk to humans, could be achieved by removal of these host species (Rudge *et al*., 2013).

1.3.2 Manipulating natural rates of transmission

Further to the genetic characterisation of parasite populations, another potentially useful approach for identifying key transmission hosts is to manipulate transmission between host species within the community and to observe the effect on disease prevalence in the host species of interest (i.e. the "target" host according to the terminology of Haydon *et al*., 2002). To such ends, applied disease control interventions (e.g., physical separation of host species, or chemotherapeutic or vaccination programmes that reduce parasite prevalence in one host species), offer useful insights (Viana *et al*., 2014). For example, fenced hunting estates in Spain have prevented contact between fenced-in wild ungulates (e.g. red deer and wild boar) and fenced-out domestic livestock for up to twenty years, but *M. bovis* infections were found within both wild and domestic animals, indicating that each group can maintain infections in the absence of transmission from the other (Gortazar *et al*., 2005). In another example, mass vaccination of cattle against Rinderpest virus eventually saw elimination of this disease from cattle and sympatric wildlife in Africa (Roeder *et al*., 2013), indicating that cattle, and not wildlife, were the key transmission hosts.

While applied control interventions offer useful insights into the nature of parasite transmission within natural multi-host communities, such quasi-experiments are undeniably limited in the broader understanding they can provide. One obvious reason for this is that interventions that increase knowledge of the disease system to optimise future control may not meet the primary goals of control interventions, i.e. to reduce the prevalence of disease in the target host species as rapidly as possible (Allen & Stankey, 2009). Interventions that may optimise general knowledge of disease transmission in the system may be impractical, too costly or even dangerous if the disease in question is a major threat to human or animal health. Furthermore, depending on the nature of the interventions being implemented, the intervention itself may act to alter the natural dynamics of parasite transmission within the system. For example, interventions often act to reduce the density of a host species putatively identified as a key transmission host, usually by culling, in order to reduce infection risk to a target host species of concern (e.g. Caley *et al*., 1999; Laurenson *et al*, 2003; Donnelly *et al*., 2006). Intensive changes to the population structure of the culled species may result in changes to

individuals' behaviour and the spatial distribution of the species, which may have confounding effects on parasite transmission (e.g. badger culling and bovine tuberculosis in Great Britain; Donnelly *et al*, 2006). Hence, such interventions that fundamentally alter the structure of the system, rather than just altering parasite transmission, may be unable to inform about rates and directions of cross-species transmission in the unperturbed state. Model host-parasite systems that are amenable to the manipulation of transmission would therefore be extremely useful for studying host-parasite dynamics without the complications that accompany applied control interventions.

1.4 Wild rodent communities as a model for studying multi-host parasite transmission

Communities of wild rodents are an important system for studying parasite transmission between host species, for a number of reasons. Firstly, a notable proportion of zoonotic parasites (22.5%) are believed to originate from rodent sources (Cleaveland *et al*., 2001), and rodent-borne diseases continue to pose a very real risk to human health (Meerburg *et al*., 2009). For example, rodents form all or part of the zoonotic reservoir for the etiological agents that cause haemorrhagic fever (hantavirus), leptospirosis (*Leptospira* spp.), bubonic plague (*Yersina pestis*) and Lyme disease (*Borrelia burgdoferi*) in humans. Therefore understanding rodent-associated transmission is of direct importance from an applied disease-control perspective.

Secondly, rodent communities, even when the parasites involved are not of medical, veterinary or conservation importance, provide highly amenable systems with which to investigate the general complexities of multi-host parasite transmission under natural conditions. Their small size and fidelity to relatively restricted spatial areas (as determined by the often spatially patchy distribution of suitable habitat; Kikkawa, 1964), means that individuals, and the populations they constitute, can be well characterised and even manipulated. Thirdly, a diversity of parasites circulates endemically within rodent communities, several of which are important models of human infections (e.g. *Heligosomoides polygyrus*, Behnke *et al*., 1999; Herpes virus,

Knowles *et al*., 2012; Cowpox virus, Crouch *et al*., 1995; *Bartonella* spp., Birtles *et al*., 1994), and studying these parasites in their natural hosts provides an invaluable context in which to interpret results of lab-based studies on them. Importantly, individual rodents are often coinfected by multiple parasite species at once (Telfer *et al*., 2010; Knowles *et al*., 2013), and many of the same parasite species appear to infect multiple rodent species in the same location (Begon *et al*., 1999; Telfer *et al*., 2007; Paziewska *et al*., 2012). Hence, wild rodents and their parasites are an ideal system for studying the community context of host-parasite interactions.

In the United Kingdom, wood mice and bank voles (*Myodes glareolus*) are abundant, widespread, and often occur in sympatry (Ashby, 1967; Crawley, 1970; Greenwood; 1978). These rodents are host to a diversity of parasites, including microparasites such as Herpes virus (Knowles *et al*., 2012), Cowpox virus (Crouch *et al*., 1995) and bacteria of the genus *Bartonella* (Birtles *et al*., 1994), and macroparasites including a range of intestinal helminths (Sharpe *et al*., 1964; Lewis & Twigg, 1972; Behnke *et al*., 1999) and ectoparasites (Whitaker, 2007). Importantly, several of what appear to be the same parasite species are reported to infect both wood mice and bank voles, and several previous studies have sought to elucidate the role of each host species in the transmission dynamics of generalist parasites.

In many cases, however, the influence of multiple host species on parasite transmission dynamics in rodent communities appears to be complex. For example, using detailed and extensive longitudinal time-series data of host abundance and individual infection status, Begon *et al*. (1999) investigated which transmission processes (within-species, between-species, or both) best explained the infection dynamics of cowpox virus (a potential zoonotic virus) in each host species population. Interestingly, although both mice and voles have a reasonably high prevalence of infection by cowpox, and occur within the same environment, the authors found a negligible role for transmission between wood mice and bank voles. While this implies that the two host species should not be considered a combined reservoir for this virus, a separate study found that transmission from bank voles might still be important for initial invasion of cowpox virus into small wood mouse populations (Begon *et al*., 2003). In another example, Telfer *et al*. (2007a) found that the prevalence of the bacterial parasite *Bartonella birtlesii* in wood mice was positively related to the previous density of bank voles,

suggesting a role for regular between-species transmission in maintaining this vector-borne parasite in the wood mouse population. However, similar evidence for between-species transmission was lacking for several other closely related *Bartonella* parasites, indicating that the occurrence of such transmission may be subject to fine-scale influences. Indeed a study of *Bartonella* infections in populations of wood mice and bank voles in Ireland further highlights the potentially complex nature of transmission within this system (Telfer *et al*., 2005). In this study, the authors found that despite an apparent absence of *Bartonella* infection in bank voles, presence of this rodent species was consistent with a reduction of *Bartonella* infection risk in sympatric wood mice, suggesting a vector-mediated "dilution" effect caused by the presence of bank voles. Given that multiple different species of *Bartonella* circulate endemically within mixed communities of wood mice and bank voles in the UK (Birtles *et al*., 2001; Telfer *et al*. 2007a), and the amenability of the overall system for study, this group of parasites offer an ideal opportunity for studying the variable roles of multiple host species in the persistence of several closely related parasite species.

1.5 *Bartonella* parasites as models for investigating multi-host parasite transmission

Bartonella species are gram-negative proteobacteria and haemoparasites of a diverse range of mammalian hosts (Breitschwerdt & Kordick, 2000). Several different species have been detected within wild rodents in the UK (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al*., 2007a and 2007b) and elsewhere across the globe (Knap *et al*., 2007; Bray *et al*., 2007; Morick *et al*., 2009; Gil *et al*., 2010; Welc-Faleçiak *et al.,* 2010; Paziewska *et al*, 2012). Infections in rodents are believed to be relatively short-lived, lasting only a few weeks (Birtles *et al*., 2001; Paziewska *et al*., 2012). During this time, primary infections in endothelial cells are thought to regularly seed infection in circulating erythrocytes (Schülein *et al*., 2001), which are then ingested and transmitted between individuals chiefly by haematophageous flea vectors (Bown *et al*., 2004; Morick *et al*., 2011), although the vector-competency of ticks has also been considered (e.g. Harrison *et al*., 2012; Reis *et al*., 2011; Telford & Wormser, 2010), but dismissed

as being unlikely in a study of wild wood mice in Northern Ireland (Harrison *et al*., 2012).

The general ecology of *Bartonella* parasites infecting wood mice and bank voles in the UK has been well characterised in several studies (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al.,* 2007a; Telfer *et al*., 2010). Interestingly, longitudinal studies have shown that *Bartonella* species differ in their seasonal dynamics and responses to the abundance of each host species (Telfer *et al*., 2007a), suggesting that the transmission events underlying persistence within host populations, and the identity of any potential key transmission hosts, may vary between these parasites. However, the mechanisms that underlie these apparent differences remain unresolved and warrant further investigation.

Here, using a range of methodologies (observational, genetic and experimental), I investigate the role of wood mice and bank voles in the transmission dynamics of several co-circulating *Bartonella* species within natural woodland communities. Specifically, I attempt to determine the net direction of any transmission between bank voles and wood mice, and identify whether either species is a key transmission host for any of these parasites. Furthermore, I explore the genetic diversity of *Bartonella* parasites and the interactions between rodent species, *Bartonella* species and flea species in order to assess how interactions between hosts, vectors and microparasites may influence rates of between-species transmission in this model host-parasite system.

1.6 Outline of data chapters

Chapter 2

I first present an analysis of *Bartonella* infection dynamics in sympatric populations of wood mice and bank voles at a woodland site in the UK. Using longitudinal data of host population and infection dynamics over two years, I ask whether there is evidence that either host species is the key transmission host for each of several *Bartonella* parasites identified within this community, by assessing the effects of host density on *Bartonella* infection risk. I also address the possibility that key host species may only be identified when considering the demographic groups of individuals most important for transmission.

Chapter 3

I then investigate the genetic diversity of *Bartonella* parasites infecting rodents across three different woodland sites, and determine whether different strains of the same *Bartonella* species are associated with different host species. In so doing, I highlight the importance of fine-scale characterisation of parasite populations in understanding the complexities of parasite persistence within multi-host communities

Chapter 4

This chapter provides a detailed characterisation of the flea community infecting wood mice and bank voles at my study sites, and the potential for specific interactions between rodent species and flea species, or flea species and *Bartonella* species (or strains), to determine the occurrence of *Bartonella* spp. transmission between wood mice and bank voles.

Chapter 5

I conclude with an experimental investigation of *Bartonella* transmission dynamics within natural communities of wood mice and bank voles at three woodland sites. I use targeted treatment of voles to manipulate the potential for between-species transmission of all *Bartonella* parasites, and investigate how this affects the incidence of the different *Bartonella* species and strains within each host species.

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Chapter 2

Assessing the role of multiple host species in determining *Bartonella* infection risk in a wild rodent community.

2.1 Introduction

Most parasites can infect more than one host species, and often appear to infect multiple sympatric species within natural communities (Cleaveland *et al*., 2001; Woolhouse *et al*. 2001; Pedersen *et al*., 2005; Begon *et al*., 1999). If parasite transmission occurs between host species, then disease persistence and an individual's risk of becoming infected will, in part, be determined by the structure of the host community and the nature of transmission within it (Holt *et al*., 2003; Dobson, 2004). This is because host species within a community are unlikely to contribute equally to parasite transmission, due to differences in abundance or underlying heterogeneities related to exposure, susceptibility and immunity (Haydon *et al*., 2002; Altizer *et al*., 2003; Kilpatrick *et al*., 2006; Streicker *et al*., 2013). Certain species may contribute disproportionately to transmission and therefore be nearly completely responsible for the persistence of a parasite within a community and the infection rates of other sympatric host species (Streicker *et al*., 2013). Identifying such "key hosts" therefore offers a means to appropriately target control interventions to maximise success (e.g. Rudge *et al*., 2013; Donnelly et al. 2006; Kaare et al. 2009).

Identifying key transmission hosts within natural communities is challenging, not least because different underlying transmission networks may result in similar emergent patterns of parasite prevalence within host populations (Fenton & Pedersen, 2005). For example, a parasite common to two host species may only be able to persist within one species due to regular spillover transmission (Antonovics *et al*., 2002; Power & Mitchell, 2004) from the other "maintenance" host species (Haydon et al 2002) (i.e. "apparent multi-host parasite"). At the other extreme, regular transmission may only occur between conspecifics of each host species, and this within-species transmission may be enough to allow independent disease persistence in both species, even in the

absence of between-species transmission (i.e. "true multi-host parasite", maintained by either species alone; Fenton & Pedersen, 2005). Importantly, these different dynamics of transmission within and between species could lead to similar patterns of prevalence in the two host populations, but for very different reasons. Cross-sectional patterns of parasite prevalence may therefore be misleading when trying to understand transmission dynamics within diverse host communities (Fenton *et al*., 2014). Consequently, greater insight may be gleaned from studying longitudinal patterns of parasite prevalence in relation to temporal changes in host community structure (e.g. Begon *et al*., 1999; Begon *et al*., 2003; Telfer *et al*., 2007a; Fenton *et al*., 2014).

Identifying key transmission hosts is further complicated by the fact that, in addition to intrinsic differences in individuals' risk of infection (e.g. due to age- or gender-related differences in immunity or risk-related behaviour; Knowles *et al*., 2012; Gouveia-Oliveira & Pedersen, 2009; Folstad & Karter, 1992; Ezenwa *et al*., 2012), a subgroup of individuals within a host population may be responsible for the majority of transmission (so-called "super-spreaders") (Woolhouse *et al*., 1997; Lloyd-Smith *et al*., 2005). For example, male-biased transmission of intestinal helminths has been demonstrated in populations of yellow-necked mice, *Apodemus flavicollis* (Ferrari *et al*., 2004; Perkins *et al*., 2008), and white-footed mice, *Peromyscus leucopus* (Luong *et al*., 2009; Grear *et al*., 2012), and female-biased transmission has been found in other rodents (e.g. common voles, *Microtus arvalis*, Sanchez *et al*., 2011). Individuals differing in body size and reproductive status may also vary in their contributions to transmission. *Ixodes ricinus* ticks were found to feed in aggregated groups particularly on large-bodied sexually mature male *A. flavicollis*, highlighting the importance of this subgroup in driving the transmission of tick-borne encephalitis virus (Perkins *et al*., 2003). Identifying the "key host" within a community may therefore only be possible if analytical frameworks consider the demographic structure of the host species populations, and test the contributions from different subgroups of individuals most crucial to parasite transmission when investigating drivers of infection risk.

Communities of woodland rodents provide an amenable host system in which to investigate the complexities of multi-host parasite transmission under natural conditions. Populations of wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*) are widespread and often sympatric in the UK (Ashby, 1967; Crawley, 1970;

Greenwood; 1978). Their small size and fidelity to relatively restricted spatial areas (as determined by the often spatially patchy distribution of suitable woodland habitat; Kikkawa, 1964), means that individuals, and the populations they constitute, can be well characterised. Furthermore, these rodents are host to a diversity of parasites (including microparasites (e.g. viruses, bacteria, protozoans) and macroparasites (e.g. helminthes, fungi)), several of which are important models of human infections (e.g. *Heligosomoides polygyrus*, Behnke *et al*., 1999; Herpes virus, Knowles *et al*., 2012; Cowpox virus, Crouch *et al*., 1995; *Bartonella* spp., Birtles *et al*., 1994). Crucially, several parasites infect both host species, therefore providing an opportunity to investigate the potential roles of between-species transmission in parasite persistence.

Bacteria of the genus *Bartonella* constitute one such group of shared parasites. These gram-negative proteobacteria are haemoparasites of a diverse range of mammalian hosts (Breitschwerdt & Kordick, 2000), and several different species have been detected within wild rodents (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al*., 2007b; Knap *et al*., 2007; Bray *et al*., 2007; Gil *et al*., 2010; Welc-Faleçiak *et al.,* 2010; Paziewska *et al*, 2012). Infections in rodents are believed to be relatively short-lived, lasting only a few weeks (Birtles *et al*., 2001; Paziewska *et al*., 2012). During this time, primary infections in endothelial cells are thought to regularly seed infection in circulating erythrocytes (Schülein *et al*., 2001), which are then ingested and transmitted between individuals chiefly by haematophageous flea vectors (Bown *et al*., 2004; Morick *et al*., 2011) (although the vector-competency of ticks has also been widely debated, e.g. Harrison *et al*., 2012; Reis *et al*., 2011; Telford & Wormser, 2010).

Several species of *Bartonella* have been found to circulate endemically within wood mouse and bank vole populations in the UK, and their general ecology has been well characterised in several studies (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al.,* 2007a; Telfer *et al*., 2010). Interestingly, longitudinal studies have shown *Bartonella* species differ in their seasonal dynamics and responses to the abundance of each host species (Telfer *et al*., 2007a). Abundance of a particular host may influence the number of infectious fleas in the community and/or patterns of host movement that determine contact rate with infectious fleas. Different responses for different *Bartonella* species suggest that the transmission events underlying persistence within host populations, and the identity of key transmission hosts, may vary between these parasites. For example,

prevalence of *B. birtlesii* in bank voles and wood mice in northwest England was shown to be positively associated with previous bank vole density (Telfer *et al*., 2007a), therefore implicating bank voles as the possible key transmission host. In contrast, prevalence of a different species, *B. taylorii,* in wood mice was associated with the density of conspecifics, but wood mouse density was not associated with bank vole prevalence, which suggests that transmission between these host species is not necessary for parasite maintenance in either host. However, in a separate study, wood mouse density was positively associated with the prevalence of *B. taylorii* infections in field voles, *Microtus agrestis* (Telfer *et al*., 2007b), suggesting that wood mice may be a key host for *B. taylorii* persistence in this other vole species. Importantly, these previous studies also highlight the difficulties of determining the direction and magnitude of transmission and the effect of community composition on infection risk within natural communities, because sometimes none of the host density effects investigated were supported as drivers of infection risk, and in other cases the densities of multiple host species were equally supported.

Here, I use longitudinal data collected over 16 months to investigate the dynamics of *Bartonella* infections within a wild community of wood mice and bank voles. By characterizing the population dynamics of these sympatric host populations over time, I aim to identify which (if either) host species is the key transmission host for each of the several *Bartonella* parasites in this community, by assessing the effects of host density on *Bartonella* infection risk. Crucially, I also detail the dynamics of different demographic subgroups of each host population, enabling their role in driving *Bartonella* transmission to be assessed independently, and in doing so, investigating whether key transmission hosts are only identifiable when particular demographic groups are considered. Furthermore, I explicitly test how intrinsic differences in individuals' likelihood of infection affect the designation of key hosts in a longitudinal study of *Bartonella* infection, thus enabling a clearer insight into the roles of each host species in driving infection risk. I discuss the results in relation to previous work in similar systems, and highlight the potential role of specific interactions between hosts, fleas and *Bartonella* parasites in driving the range of host-*Bartonella* relationships observed.

2.2 Methods

2.2.1 Field methods and rodent community characterisation

Wood mice and bank voles were live-trapped during 2010 and 2011 within Manor Wood (MW), an area of mixed deciduous woodland in northwest England (N 53.3301°, E -3.0516°). Sherman traps (Alana Ecology, UK), baited with mixed grain and carrot, were deployed in pairs at 10m intervals within 70m x 70m trapping grids (1 grid = 128 traps). Data were collected from a single trapping grid in 2010 (MW1), and from two trapping grids, in 2011 (MW1, MW2). Trapping sessions took place every 4 weeks from May to November in 2010 (7 trapping sessions), from May to December on MW1 in 2011 (8 trapping sessions), and from June to December (7 trapping sessions) on MW2 in 2011. Traps were set over night and checked the following morning on 3 consecutive occasions within each trapping session.

Animals were given a subcutaneous electronic passive induced transponder (PIT-tag) (AVID MicroChips, UK) with a unique 9-digit identification number upon first capture, so that individuals could be longitudinally followed throughout their life and population densities could be estimated. On first capture within each trapping session, all individuals were identified by PIT-tag, sexed, weighed to the nearest 0.5g and measured in length from their nose to their tail base. Individuals were assigned to one of three age categories based on pelage colour in the first instance, with body mass used as a secondary trait where pelage was inconclusive (Juvenile if <12g, Sub-Adult if 12-16g, Adult if >16g). Reproductive condition of animals was also assessed. Females were considered reproductively active if they had a perforate vagina, were obviously pregnant, or showing signs of lactation (obvious nipples), while males were reproductively active if their testes had descended from their abdomen. A small blood sample (~30μL) was also taken from the tail tip on first capture within each trapping session for subsequent diagnosis and identification of *Bartonella* infections. All animals were then released at the site of capture.

Total population densities of each host species were calculated using minimum number known alive (MNKA) on each trapping grid in each trapping session – a metric known

to be highly correlated with other estimates of population density (Clotfelter *et al*., 2007). Densities of male and female wood mice and bank voles were also estimated separately using the same method. As the reproductive status of an individual is not constant through time, the density of reproductively active and reproductively inactive individuals of each host species were estimated as the numbers within each group that were captured per grid in each session.

2.2.2 Detecting and identifying *Bartonella* spp. infections

Blood samples collected from the field were centrifuged at 16,000 G for 10 minutes to separate blood pellets (containing cells) from sera, and then frozen at -20°C until further processing.

2.2.2.1 Blood DNA extraction and quantification

DNA was extracted from blood pellets using DNAzol BD reagent© (Invitrogen, UK). 5µL of the blood sample, rehydrated in ultrapure water, was added to 200µL of DNAzol BD reagent, along with 2µL GenElute-LPA polyacrylamide carrier (Sigma-Aldrich, UK). The solution was vortexed and incubated at room temperature for 15 minutes, before the addition of 100µL of 100% isopropanol to precipitate the DNA. Following a further 15-minute incubation at room temperature, solutions were centrifuged for 5 minutes at 5000xG to pellet the DNA. The DNA pellets were washed twice in 100% ethanol and re-suspended in 50µL TE buffer. All DNA extractions were quantified using the Qubit™ double-stranded DNA high-sensitivity assay (Invitrogen, UK), according to the manufacturer's guidelines to measure the quantity of DNA in each sample. We used this metric as a covariate in all statistical analyses to account for the variability in DNA quantity between samples, as it may affect the ability to detect parasite infection in subsequent assays.

2.2.2.2 PCR assay for the detection of *Bartonella* spp. infections

Extracted DNA was used as a template in a semi-nested polymerase chain reaction that targeted a genus-specific 300-500bp fragment of the 16S-23S internal transcribed spacer region using the primers of Telfer *et al*. (2005), which are reproduced in their 5'→3' formats below:

Round 1
Forward primer (big-F): TTG ATA AGC GTG AGG TC
Reverse primer (bog-R): TGC AAA GCA GGT GCT CTC CCA

Round 2
Forward primer (big-F): as in round 1
Reverse primer (big-R): TCC CAG CTG AGC TAC G

First round PCR mixtures comprised 2µL DNA, 12.5µL Biomix Red PCR Readymix (Bioline), 8µL ultrapure water, 2µL 25mM $MgCl_2$, and 0.25µL each of 10µM big-F and bog-R primers. The mixture was exposed to the following thermal cycle: 96°C for 3 minutes, 13 x [96°C for 10s, 61°C for 10s decreasing by 0.5°C each cycle, 72°C for 50s], 8 x [96°C for 10s, 55°C for 10s, 70°C for 50s].

Second round PCR mixtures comprised 1µL first round PCR product, 12.5µL Biomix Red PCR Readymix, 9µL ultrapure water, 2µL 25mM $MgCl_2$, and 0.25µL each of 10µM big-F and big-R primers. The mixture was exposed to the following thermal cycle: 96°C for 3 minutes, 13 x [96°C for 10s, 61°C for 10s decreasing by 0.5°C each cycle, 72°C for 50s], 22 x [96°C for 10s, 55°C for 10s, 70°C for 50s]. Second round PCR products were visualised on a 2% (w/v) agarose gel stained with Ethidium Bromide (EthBr), run for 30 minutes at 120V. Samples positive for *Bartonella* spp. infection were identified if a band occurred within the 300-500bp size-range.

The region of DNA targeted with this PCR assay varies in length between different species of *Bartonella* (Roux & Raoult, 1995; Birtles *et al*., 2000; Houpikian & Raoult, 2001), and species-level infection can be determined by assessing the length of the PCR amplification product (as in Telfer *et al*., 2005, Telfer *et al*., 2007a). All positive samples were therefore subsequently visualised on a more concentrated 3% (w/v) agarose gel stained with EthBr, run for 2.5 hours at 120V, to more accurately determine amplicon size. Details of *Bartonella* species identification based on amplicon length are given in Table 2.1.

Note that certain *Bartonella* species are indistinguishable by their amplicon length, such as *B. rudakovii* and BGA amplicons, which are are both ca. 480bp, and *B. doshiae* and *B. doshiae*-like, which are both ca. 300bp. However, subsequent DNA sequencing of a subset of PCR products confirmed strict host-specificity of these infections, such that amplicons of approximately 480bp in length from bank voles were always identified as *B. rudakovii*, whereas those from wood mice were always BGA (details given in Chapter 3; 33/53 and 66/150 PCR products of ca. 480bp in size were sequenced from wood mice and bank voles respectively). Similarly, amplicons of ca. 300bp from bank voles were always identified as *B. doshiae*, while from wood mice were always *B. doshiae*-like (50/154 and 8/12 PCR products of ca. 300bp in size were sequenced from wood mice and bank voles respectively). This host-specificity is therefore assumed in the following analyses of infection patterns.

Table 2.1: Classification of *Bartonella* spp. infections according to length of the amplicon produced by a PCR targeting a fragment of the 16S-23S internal transcribed spacer region, following Telfer *et al*., 2005.

Approximate PCR amplicon size (bp)	***Bartonella* species**
300	*B. doshiae* or *B. doshiae*-like
320	*B. grahamii*
350	*B. taylorii*
370	*B. birtlesii*
480	*B. rudakovii* or BGA

2.2.3 Statistical methods to determine *Bartonella* spp. infection risk

The prevalence of *B. doshiae* in bank voles and BGA in wood mice were both very low and the statistical models detailed below failed to converge. As a result, statistical analyses of infection risk here are restricted to *B. grahamii*, *B. taylorii* and *B. birtlesii* in both hosts, *B. doshiae*-like in wood mice only, and *B. rudakovii* in bank voles only.

I used generalized linear models (GLMs; with logit link for binomial errors) for each host species separately, to determine the key factors associated with the risk of infection for each *Bartonella* species. In each case, two complementary approaches were used. The first modeled population level infection risk, following the approach used by Telfer *et al*. (2007a), with the binomial response variable being the number of hosts infected and uninfected within each trapping session on each grid. The second approach modeled infection risk at the individual level, with the response variable as the binomial infection status (infected or uninfected) of each individual in the data set. Both approaches were used to evaluate the importance of extrinsic contributors to infection risk (see details below); however the latter approach also allowed for the investigation of intrinsic risk factors related to individual characteristics (see details below), which may confound any apparent extrinsic risk factors.

2.2.3.1 Extrinsic determinants of infection risk

To account for temporal fluctuations in environmental conditions that may impact on the population dynamics and vector capacity of fleas, I investigated sampling year (as a factor with 2 levels) and a two-part sinusoidal term representing annual seasonality (see Appendix 2.1) as extrinsic effects in all models. The roles of each host species were also assessed as extrinsic drivers of risk, by investigating the explanatory power of several different covariates describing population densities for each host species: total density, male density, female density, number of sexually active individuals and number of sexually inactive individuals. The densities of different age groups within populations

were not investigated due to the relatively small number of young individuals within the data set.

Both current (*t*) and lagged (*t*-2 months) measures of host population densities were explored, as effects on infection risk may be delayed. The relatively short length of the time series limited the investigation of lagged host densities; density effects with a greater time lag were not possible without a significant loss of power (with a lag of 2 months, sample sizes were reduced to $N = 262$ wood mice and $N=265$ bank voles). A lag of t-1 month was also omitted due to the high degree of temporal correlation between population densities separated by a single month (see Appendix 2.3).

2.2.3.2 Intrinsic determinants of infection risk

To account for variation between individuals in their risk of infection, regardless of extrinsic infection risk, I modeled age (a factor with two levels: Young [juvenile or sub-adult], or Adult), sex (a factor with two levels: Male or Female) and reproductive status (a factor with two levels: Active or Not active), and a two-way interaction between sex and reproductive status. As these explanatory factors rely on information at the individual level, these factors could only be investigated using the individual level modeling approach.

2.2.3.3 Modeling framework

The contribution of each extrinsic factor to infection risk was first investigated at the population level. A suite of candidate models was constructed, which represented all possible additive combinations of the extrinsic effects as described above. Due to the high degree of correlation between different measures of population densities within and between host species and across time points (see Appendix 2.3), the explanatory power of each population density metric was investigated independently within separate candidate models, in an attempt to avoid problems related to collinearity of explanatory variables (Mac Nally, 2000). Therefore each candidate model included year, seasonality and one of the population density terms. It was not possible to investigate interactions

between extrinsic factors, as asymmetry of the data (unequal sampling across years and some inconsistent sampling across trapping sessions) caused non-convergence of models when such terms were included.

As data from the same individual were often collected for several trapping sessions, population infection prevalence through time is not independent. To account for this repeated sampling and potential temporal autocorrelation, infection prevalence of the focal *Bartonella* spp. within the focal host species population in the previous trapping session was also included as a fixed effect in all models of population-level infection risk (following Telfer et al., 2007a)). The explanatory power of all candidate models was compared using their AICc values (Akaikes Information Criterion, adjusted for small sample sizes) (Burnham & Anderson, 2002; Johnson & Omland, 2004), and the best model was identified as the one that was most parsimonious within the top 2 AICc and with an AICc at least 2 units below that of a null model containing no terms (Burnham & Anderson, 2002). If more than one model fitted these criteria, then they were regarded as equally good fits. Factors and/or covariates contained within this/these best model(s) were therefore evidenced as potential drivers of population infection risk.

Modeling of infection risk at the individual level used a similar framework, but also investigated the potential intrinsic drivers of infection risk as outlined above, alongside extrinsic drivers. With the inclusion of these additional effects, the number of candidate models increased to >800, therefore a two-stage model selection process was introduced. The first stage identified which of the intrinsic factors were potential contributors to infection risk. A suite of candidate models was constructed, representing all possible additive combinations of intrinsic factors (age, sex, reproductive status) and a two-way interaction between sex and reproductive status. The best model was identified as the one that was most parsimonious within the top 2AICc and with an AICc at least 2 units below that of a null model (Burnham & Anderson, 2002), and any intrinsic factors within this best stage one model were then included in a second stage of modeling.

The second stage of modeling aimed to determine whether any of the extrinsic factors contributed to infection risk at the individual level after the intrinsic parameters were included. Thus here the suite of candidate models represented all combinations of

extrinsic terms (year, seasonality, plus a single population density term) and the intrinsic terms (including any interactions) that were supported in stage one (n = 84 – 420 models). I included DNA concentration in all models, as the probability of detecting microparasite infections in rodent blood is known to increase with increasing DNA concentration (Knowles *et al*., 2013). The best stage two model(s) was identified based on AICc comparisons as earlier described, and any factors/covariates therein were supported as drivers of infection risk at the individual level.

2.2.3.4 Bootstrapping and mixed models to address non-independence of data

To further address the problem of non-independence within the data set arising from multiple captures of some individuals, for models of individual-level infection risk I first attempted to use generalised linear mixed models (GLMMs), incorporating individual PIT-tag number as a random effect (Paterson & Lello, 2003; Bolker *et el*., 2009). However, models that included this random effect often failed to converge. I therefore used a bootstrapping methodology instead (Fenton *et al.,* 2014) for both the population level and individual level approaches. In each case, for 100 bootstrap replications, a single capture per individual was selected at random from the full data set. The modelling procedures as described above were then performed on these smaller data sets, and the best model(s) for each simulation was identified. The percentage of bootstrap simulations for which each candidate model was identified as the best fit was then calculated and used to infer the level of support for each model. Terms within models that were supported in more than 50% of bootstraps, or terms consistently appearing in models whose combined bootstrap support exceeded 50%, were considered as having a high level of support and therefore important predictors of infection (Fenton *et al*., 2014). Note that multiple models were often identified as equally good fits for a bootstrap simulation and therefore, when summed, the percentage support for candidate models identified using the full data set often exceeded 100%.

For models of infection risk at the population level, the number of wood mice and bank voles infected and not infected with each *Bartonella* species for each grid-session combination were calculated for each simulated data set before being used as a response in the models. Prevalence in the previous month was not included in these bootstrap

population-level models, as temporal autocorrelation of population prevalence was no longer a problem now that only a single data point per individual was used. For models of infection risk at the individual level, the bootstrapping procedure was conducted in two stages. Stage one models were bootstrapped first, and only intrinsic effects with a high level of support (as defined above) were incorporated in the subsequent stage two modeling and bootstrapping procedure, which was conducted on a new set of 100 simulated data sets.

I also attempted to run models of both population- and individual-level infection risk as GLMMs (using the lme4 package in R), with all fixed effects as above, and with trapping session as a factor with 16 levels (each of 8 independent trapping sessions across 2 years) as a random effect. This was to address the possibility that populations sampled at the same time are correlated due to shared experiences unrelated to any of our recorded variables (Paterson & Lello, 2003). This specifically attempted to account for temporal correlation between populations sampled during the same sessions on MW1 and MW2 during 2011. However, there were problems estimating the size of this random effect, perhaps because relatively few individuals were infected within certain sessions. Models including this random effect therefore rarely converged. I therefore present only the results for the GLMs. All GLMs were fitted using Laplace approximation to maximum likelihood estimation, using R (Version 2.14.2)

2.3 Results

2.3.1 Rodent community dynamics

247 wood mice and 298 bank voles were caught in total in 2010-11 on the 2 trapping grids, resulting in a total of 571 wood mouse captures and 639 bank vole captures. The majority of individuals were caught within just one trapping session (59% of wood mice and 58% of bank voles), although some individuals of both species appear many times across the data set, with examples of both hosts being captured multiple times across the 16-month period (Figure 2.1). The mean number of captures per wood mouse was 1.80 ± 0.08[SE] and per bank vole was 1.70 ± 0.06[SE], while the mean number of captures per individual caught more than once was 2.93 ± 1.13 for wood mice and 2.64 ± 0.09 for bank voles.

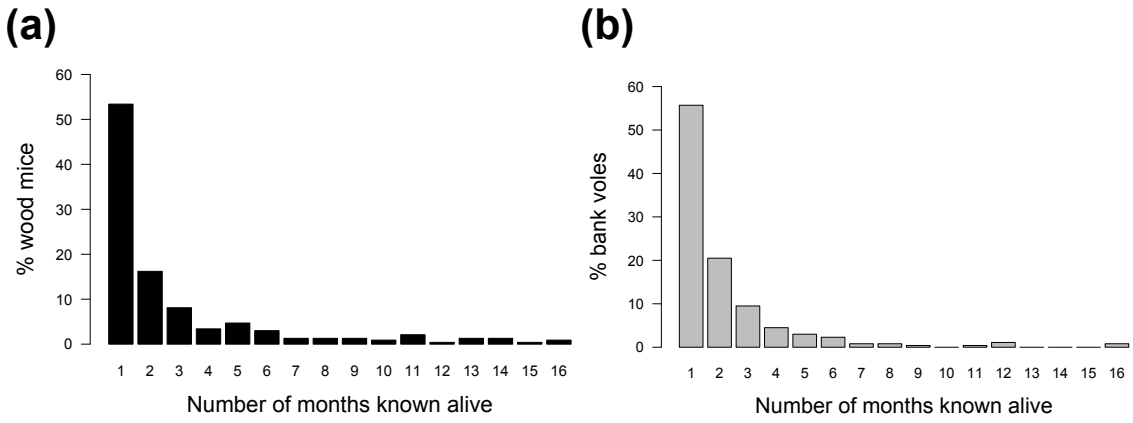

Figure 2.1: Frequency distributions of the number of months known alive for (a) wood mice and (b) bank voles, throughout the study period.

Overall capture numbers of both host species varied in space and time (Table 2.2). On MW1 more individuals of both host species were caught in 2011 (117 wood mice and 103 bank voles) compared to 2010 (67 wood mice and 71 bank voles). However, in 2011, relative capture numbers of wood mice and bank voles varied across the two trapping grids: comparable numbers of each host species were captured on MW1, with a slight wood mouse bias (ratio of wood mice: bank voles = 1.14), but bank voles on

MW2 far exceeded wood mice (ratio of wood mice: bank voles = 0.62). Furthermore, across both years and grids, wood mice displayed relatively stable seasonal dynamics (average ratio of highest to lowest densities = 3.9 ± 2.88 SD), whereas bank vole dynamics were much more variable (average ratio of highest to lowest densities = 17.8 ± 8.09 SD) (Figure 2.2). Bank vole population densities were positively correlated with greater proportional representation of bank voles within the sympatric rodent community (Spearman's rank correlation, $r = 0.82$, $p < 0.001$; Figure 2.3a), but this relationship was not evident for wood mouse population densities ($r = -0.20$, $p = 0.35$; Figure 2.3b). Overall, total rodent community densities were positively correlated with both wood mouse ($r = 0.86$, $p < 0.001$; Figure 3c) and bank vole ($r = 0.87$, $p < 0.001$; Figure 2.3d) population densities. However, as bank vole densities reached greater numbers (maximum = 73) compared to wood mouse densities (maximum = 34), total rodent community densities >60 were independent of wood mouse densities (Figure 2.3c), and were instead driven solely by high bank vole abundances (Figure 2.3d). Overall, densities of wood mice and bank voles were positively correlated ($r = 0.58$, $p < 0.01$), although the strength of the correlation varied across years and trapping grids (Figure 2.3e).

Table 2.2: Number of individuals and captures of each host species on each trapping grid in each year of study, and in total. In 2011, some individuals were caught on both trapping grids (n=20), and some individuals were caught in both years (n=33), and so are counted as unique individuals in each grid-year category for which they were caught. Total number of individuals of each host species is therefore less than the sum of the number of individuals for each grid-year category.

Grid	Year	Number of individuals		Number of captures	
		WM	BV	WM	BV
MW1	2010	67	71	148	174
MW1	2011	117	103	247	197
MW2	2011	88	141	176	268
Total		247	298	571	639

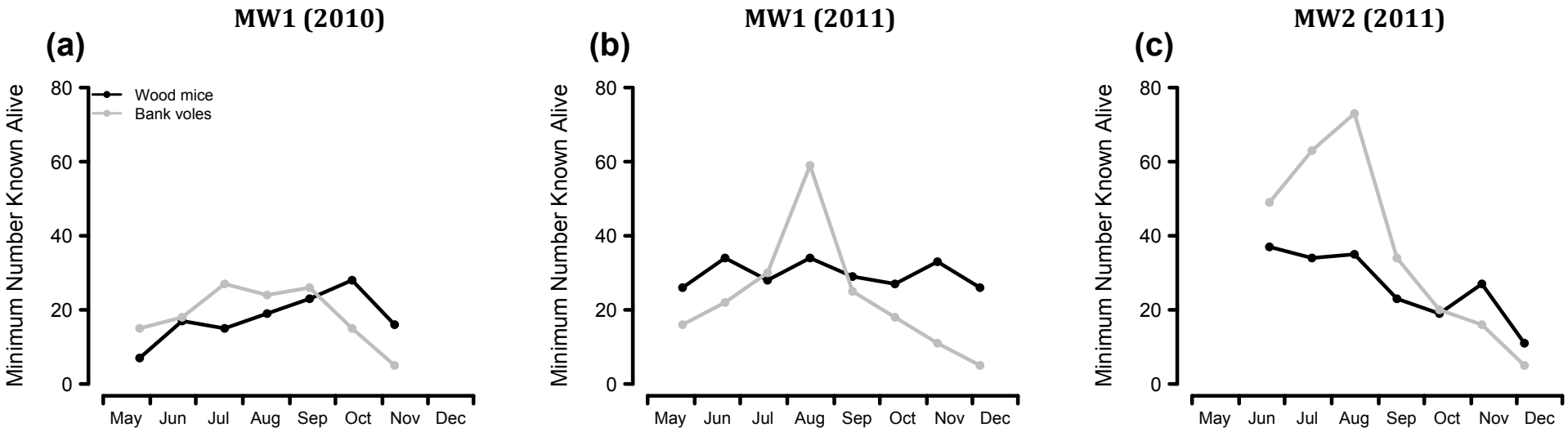

Figure 2.2: Seasonal changes in wood mouse (black lines) and bank vole (grey lines) density, measured as the minimum number known alive (MNKA), on (a) MW1 in 2010 (b) MW1 in 2011 and (c) MW2 in 2011.

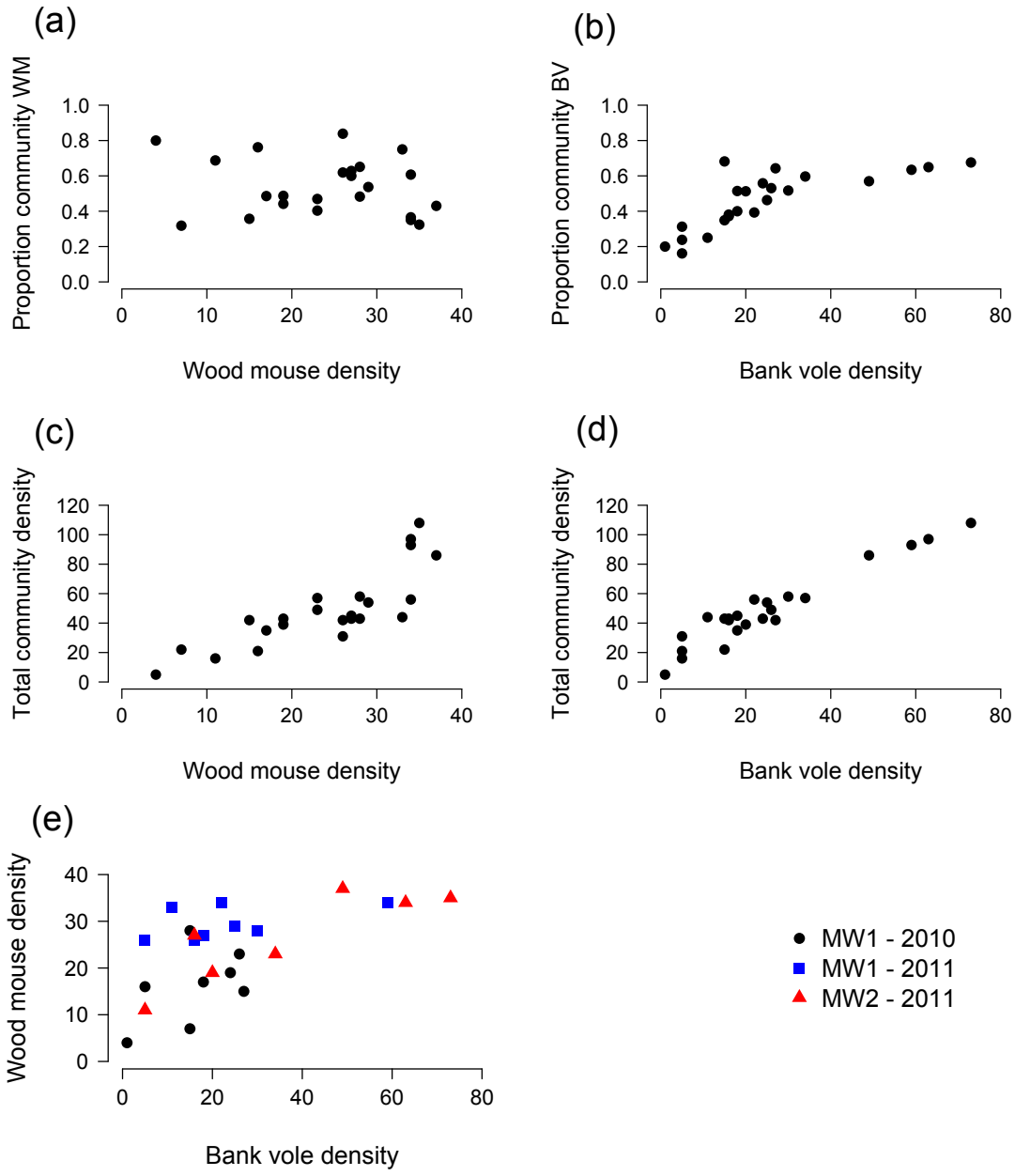

Figure 2.3: Relationship between (a) wood mouse density and the proportion of the rodent community that comprised wood mice (Spearman's rank correlation, r = -0.20, p = 0.35); (b) bank vole density and the proportion of the rodent community that comprised bank voles (r = 0.82, p < 0.001); (c) wood mouse density and total rodent community density (r = 0.86, p < 0.001; (d) bank vole density and total rodent community density (r = 0.87, p < 0.001); (e) bank vole density and wood mice density overall (r = 0.58, p <0.01) and on MW1 in 2010 (r = 0.38, p = 0.35), MW1 in 2011 (r = 0.57, p = 0.14) and MW2 in 2011 (r = 0.79, p = 0.05).

There was variation in the population dynamics of different demographic groups, across time and between the two host species (Figure 2.4 – 2.6). The densities of many of these demographic groups were correlated, both within host species (62% and 100% of pair-wise correlations were significant for wood mouse and bank vole densities respectively) and between host species (57% of densities were correlated) (see Appendix 2.3).

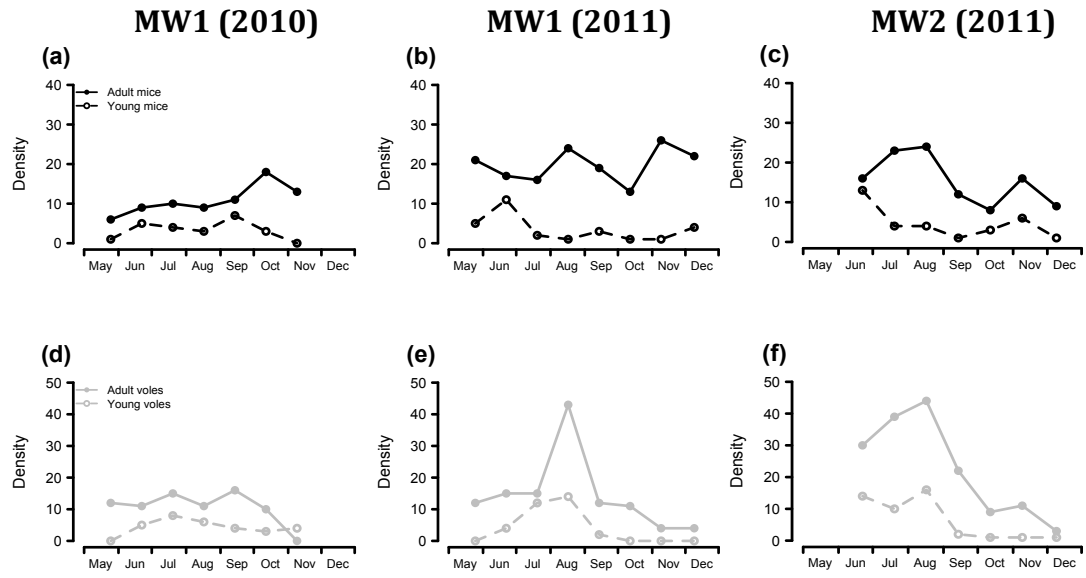

Figure 2.4: Population dynamics of young (juvenile and sub-adult) and adult wood mice (a-c) and bank voles (d-f) across trapping grids and years. Densities of adults were consistently higher than those of young individuals for both host species, but young individuals were more sustained in their presence throughout 2010 compared to 2011. Peaks in the abundance of young individuals coincided with peaks in reproductive activity in both species (Figure 2.5). Data from different trapping grids and years are arranged in columns: Left-hand column = MW1 in 2010, Middle column = MW1 in 2011, Right-hand column = MW2 in 2011.

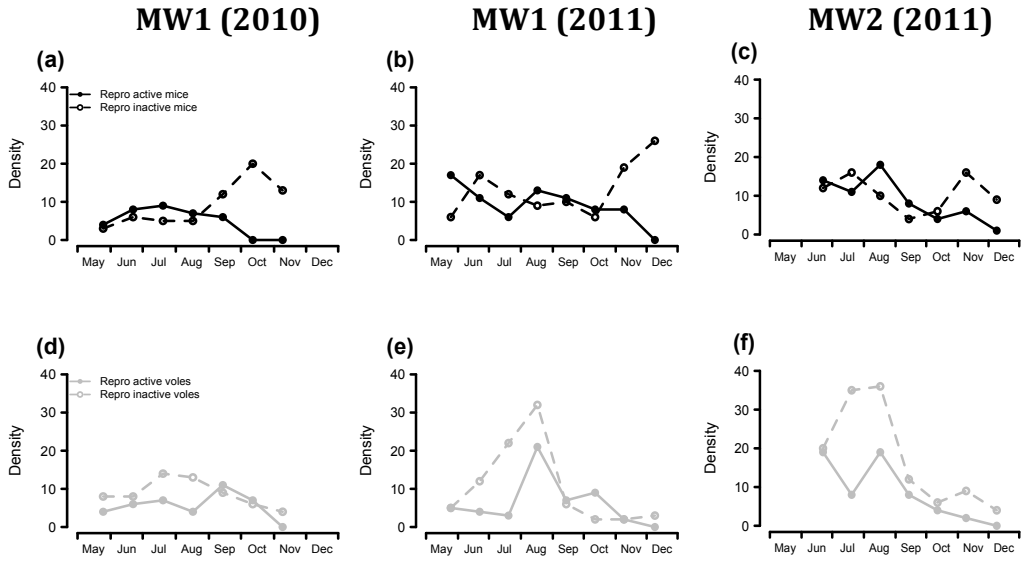

Figure 2.5: Population dynamics of reproductively active and reproductively inactive wood mice (a-c) and bank voles (d-f) across trapping grids and years. In general, peaks of reproductive activity in bank voles occurred later in the year and were less sustained than in wood mice. Data from different trapping grids and years are arranged in columns: Left-hand column = MW1 in 2010, Middle column = MW1 in 2011, Right-hand column = MW2 in 2011.

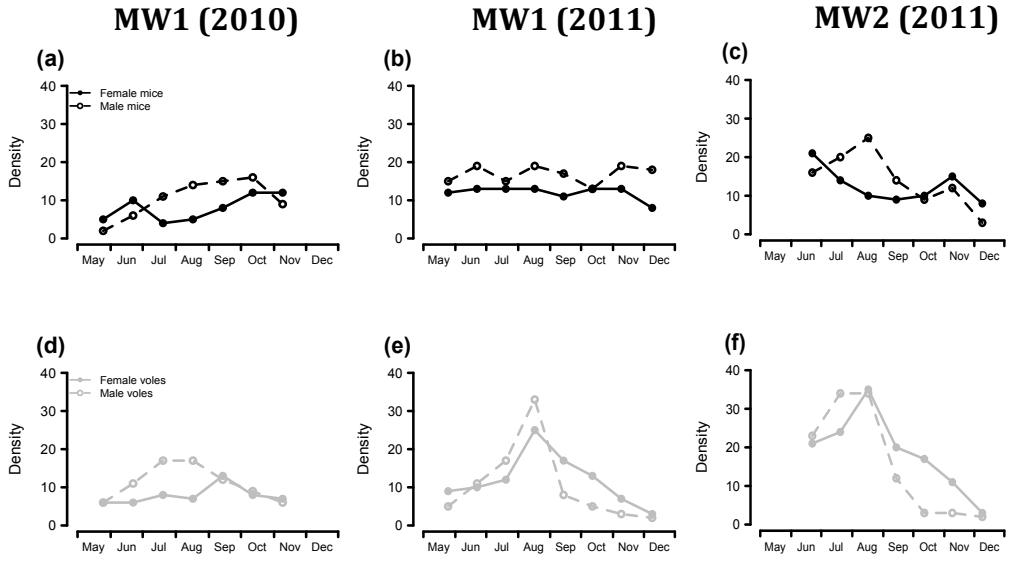

Figure 2.6: Population dynamics of male and female wood mice (a-c) and bank voles (d-f) across trapping grids and years. The sex ratio of both hosts fluctuated through time, but populations were apparently male-biased for the majority of the study period, especially in wood mice. Data from different trapping grids and years are arranged in columns: Left-hand column = MW1 in 2010, Middle column = MW1 in 2011, Right-hand column = MW2 in 2011.

2.3.2 Patterns of *Bartonella* spp. infection

374 blood samples were taken from 214 wood mice and 397 blood samples were taken from 249 bank voles. The median number of samples per individual was 1 in both species, although multiple samples were taken from 42.5% of wood mice and 35.3% of bank voles with up to 8 and 7 samples per individual respectively (Figure 2.7).

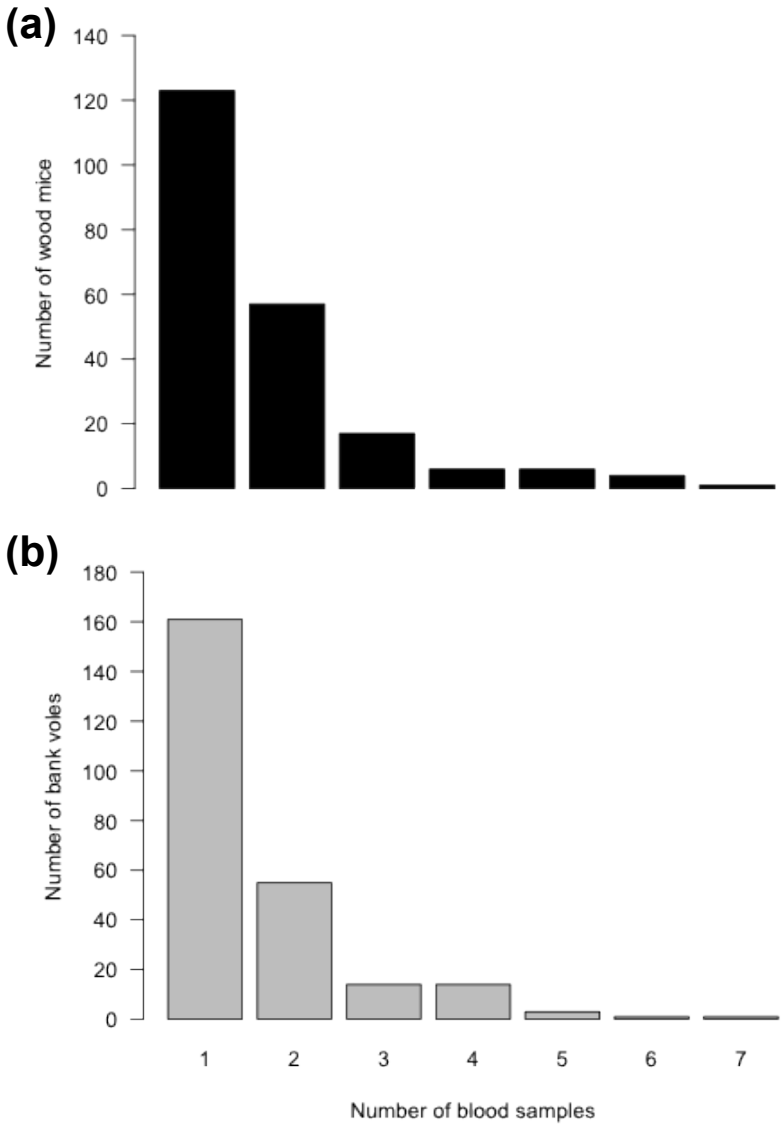

Figure 2.7: Frequency distribution showing the number of blood samples taken per individual (a) wood mice and (b) bank voles throughout the study period.

57.5% of wood mice and 60.6% of bank voles were infected with *Bartonella* at least once throughout the study period, and multiple species of *Bartonella* were found in 10.4% of wood mouse samples (*n*=39) and 7.6% of bank vole samples (*n*=30), indicating the presence of co-infections. *B. rudakovii* was only found in bank voles, while *B. doshiae*-like and BGA was only found in wood mice. Of the *Bartonella* species found in both hosts, *B. taylorii* was more prevalent in wood mice (35%), while *B. grahamii* (28%) and *B. birtlesii* (28%) were more prevalent in bank voles (Table 2.3).

Table 2.3: Proportion of individuals that tested positive for each *Bartonella* species at least once during the course of the study.

***Bartonella* species**	**Wood Mice *n* = 214**	**Bank Voles *n* = 249**
B. doshiae-like	0.18 (*n* = 39)	0 (*n* = 0)
B. grahamii	0.14 (*n* = 30)	0.28 (*n* = 70)
B. taylorii	0.35 (*n* = 75)	0.16 (*n* = 39)
B. birtlesii	0.17 (*n* = 37)	0.28 (*n* = 69)
B. rudakovii	0 (*n* = 0)	0.11 (*n* = 28)
BGA	0.01 (*n* = 2)	0 (*n* = 0)

Raw time-series data showing infection prevalence of each *Bartonella* spp. in populations of each host species demonstrate seasonal variation in infection prevalence (Figure 2.8). However, this variation was not consistent across years or trapping grids.

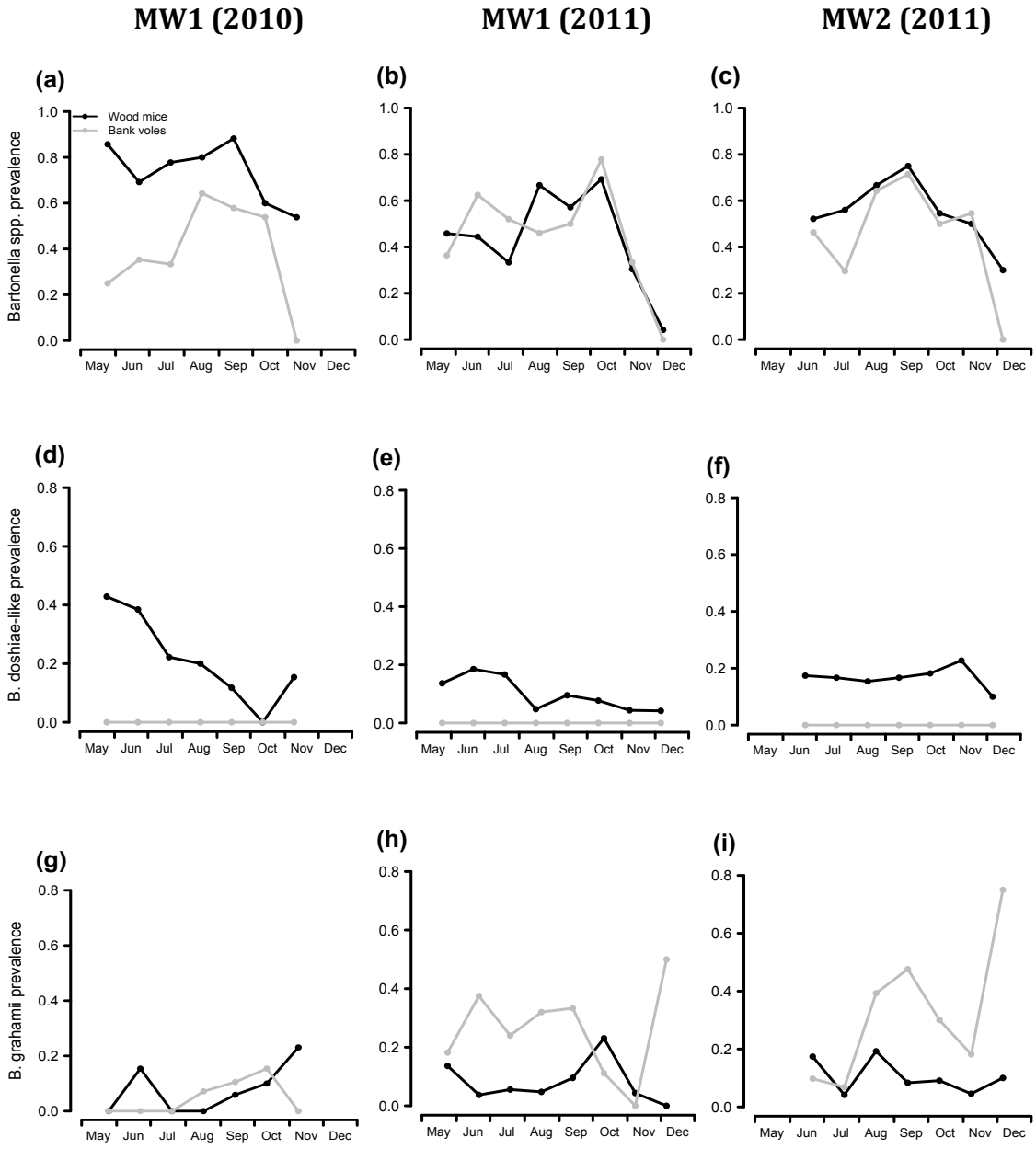

Figure 2.8: *Bartonella* infection prevalence in wood mice (black lines) and bank voles (grey lines) across both trapping grids and years of study. a-c: All *Bartonella* spp. combined, d-f: *B. doshiae*-like, g-i: *B. grahamii* j-l: *B. taylorii*, m-o: *B. birtlesii*, p-r: *B. rudakovii.* Seasonal peaks of *B. doshiae*-like in wood mice generally occurred earlier in the year (May) compared to peaks of all other *Bartonella* species in either host. Prevalence of *B. taylorii* was generally much higher in wood mice than bank voles throughout both years and on both grids. *B. birtlesii* prevalence was only consistently higher in bank voles on MW1 and not MW2. *B. grahamii* was generally higher in bank voles throughout 2011 on both grids, but prevalence in each host was similar in 2010. Prevalence of *B. rudakovii* in bank voles was generally higher in 2011 compared to 2010. Data from different trapping grids and years are arranged in columns: Left-hand column = MW1 in 2010, Middle column = MW1 in 2011, Right-hand column = MW2 in 2011. Figure continued on next page.

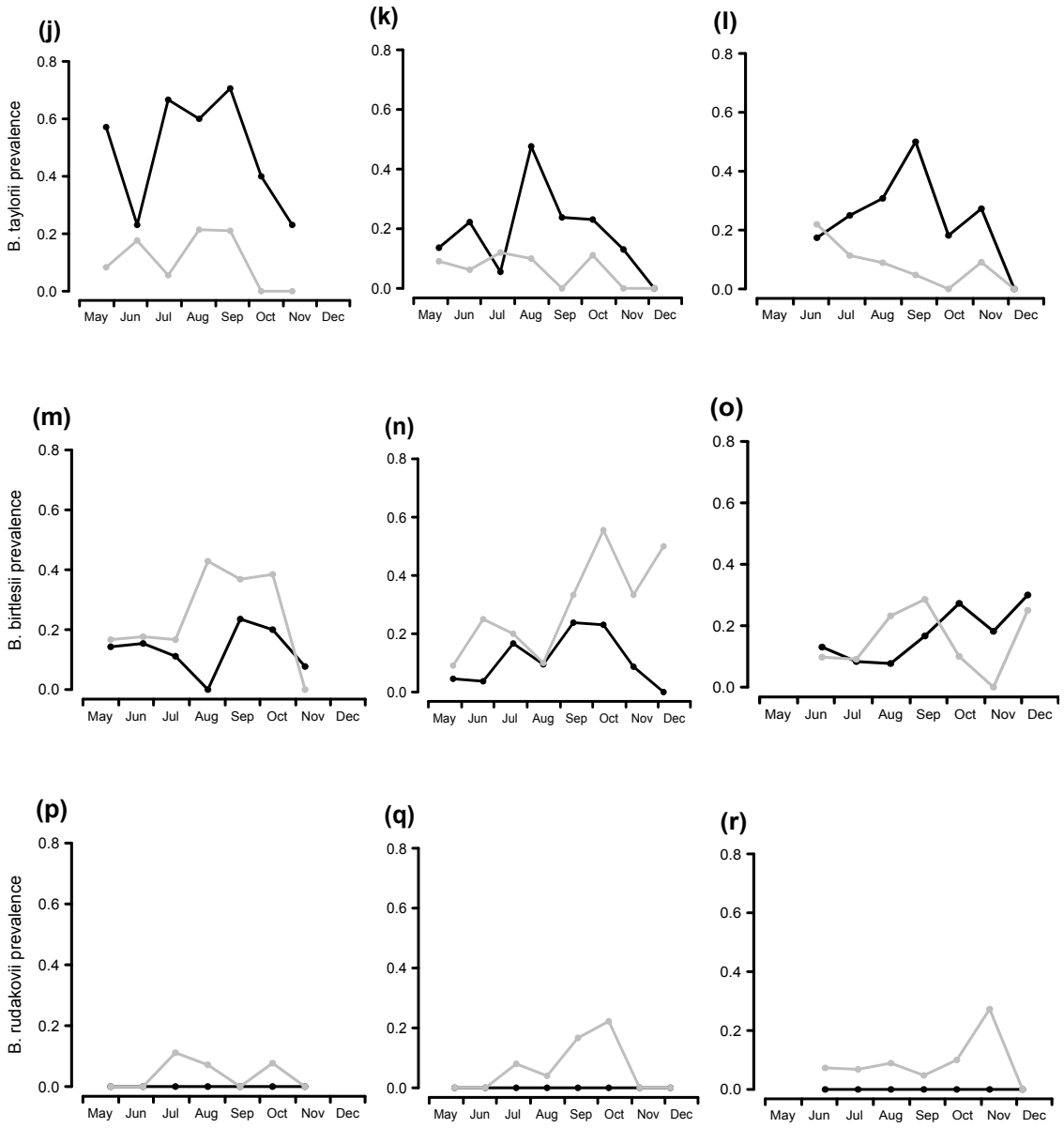

Figure 2.8: Continued from previous page.

2.3.3 Statistical models of *Bartonella* spp. infection risk

Risk of infection with each *Bartonella* species was modeled for each host species at both the population level and individual level. A summary of the effects that were supported by both approaches, and supported in more than 50% of bootstrap simulations, is given in Table 2.4. Below I present the results from all statistical approaches for each *Bartonella* species in turn. Full model selection tables are presented in tables in the relevant host-parasite sections and parameter estimates for the most supported best-fit models are given in Appendix 2.4.

Table 2.4: A summary of the best-fit models of infection risk for each *Bartonella* spp. in each host species. Only extrinsic effects supported by both individual- and population-level modeling approaches, and substantiated by the bootstrapping procedure, are presented. Intrinsic variables are in blue text; extrinsic variables are in black text. All parameter estimates (for individual- and population-level models) are presented in the appendix, and given in the text where appropriate. The direction of supported effects are given in brackets: ">" denotes differences in risk between levels of a factor, and "+/-" denotes the direction of covariate effects.

***Bartonella* spp.**	**The best fit model(s) of infection risk**	
	Wood mice	**Bank voles**
***B. doshiae*-like**	Age (adult > young) Reproductive status (active > inactive)	
B. grahamii	Male bank vole density t-2 (+)	Year (2011 > 2010)† Total wood mouse density t-2 (+)†
B. taylorii	Seasonal component (summer peak) Total wood mouse density t-2 (-)	Age (adult > young) Sex (male > female)
B. birtlesii	None	Sex*Reproductive status (male > female, but only when reproductively active)
B. rudakovii		None

† These explanatory terms received equal support within two separate candidate models.

2.3.3.1 *B. doshiae*-like (wood mice)

The best-fit model of population infection risk for *B. doshiae*-like in wood mice included only a positive effect of *B. doshiae*-like prevalence in the previous month (estimated coefficient = 4.208 ± 2.334[SE]) (Table 2.5a). No extrinsic effects were supported using the full data set, and a null model with no effects was supported in 67% of the bootstrap simulations.

The best-fit stage one model of individual infection risk included intrinsic effects of both age and reproductive condition (Table 2.5b). Bootstrap support for a single model containing both of these terms was lacking, but relatively high support was found for each independently (a model containing just an age effect was a best-fit model in 48% of bootstrap simulations; a model containing just an effect of reproductive condition in 42% of bootstrap simulations), and therefore both intrinsic terms were included in the second stage of modeling.

The best-fit stage two model of individual infection risk did not support any extrinsic effects. Instead, it included only the intrinsic effects of age and reproductive condition (increased risk of *B. doshiae*-like in adult wood mice [16.301 ± 1029; Figure 2.9a; 0/40 young individuals were infected with *B. doshiae*-like]; and reproductively active wood mice [0.921 ± 0.405; Figure 2.9b]), and DNA concentration (0.012 ± 0.170) (Table 2.5c). Bootstrap simulations identified 65 different models as best-fit models at least once, none of which matched this model. However, one or both of these intrinsic terms were included in all of these best-fit bootstrap models (while no combinations of extrinsic terms was ever supported in more than 37% of simulations).

Table 2.5: Selection tables and bootstrap support for models of *B. doshiae*-like infection risk in wood mice. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago. Table continued on next page.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(a) Population-level risk					
None	**2**	**51.47**	**0**	**0**	**67**
Female BV density t_{-2}	3	52.58	1.11	1.11	0
Total BV density t_{-2}	3	53.01	1.54	1.54	0
#Repro inactive WM t	3	53.17	1.70	1.70	11
#Repro active BV t_{-2}	3	53.39	1.92	1.92	0
(b) Individual-level risk: Intrinsic effects					
Age + Reproductive condition	**4**	**178.65**	**0**	**-11.84**	**16†**
Age + Sex + Reproductive condition	5	179.67	1.02	-10.82	0
None	2	190.49	11.84	0	0
Age	3				48†
Reproductive condition	3				42†

† Bootstrap support for a single model containing both Age and Reproductive condition terms was lacking, but relatively high support was found for each independently therefore both intrinsic-related terms were included in stage 2 models.

Table 2.5: Continued from previous page.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(c) Individual-level risk: Extrinsic effects					
(Intrinsic terms: Age + Repro. condition)					
Age + Repro + Total WM density t	5	177.66	0	-12.82	3
Age + Repro + Male WM density t	5	177.95	0.29	-12.54	19
Age + Repro + #Repro inactive WM t_{-2}	5	178.23	0.56	-12.26	0
Age + Repro	**4**	**178.65**	**0.98**	**-11.84**	**0***
Age + Repro + Total WM density t_{-2}	5	179.12	1.46	-11.37	13
Age + Repro + #Repro active BV t	5	179.27	1.61	-11.22	2
None	2	190.49	11.84	0	0

* There was no bootstrap support for a model containing just effects of intrinsic Age and Reproductive condition. However, all 65 models supported by bootstrap simulations (whose bootstrap support ranged from 1%-37%) included one or both of these intrinsic terms, even though no combination of extrinsic terms was ever supported in more than 37% of simulations.

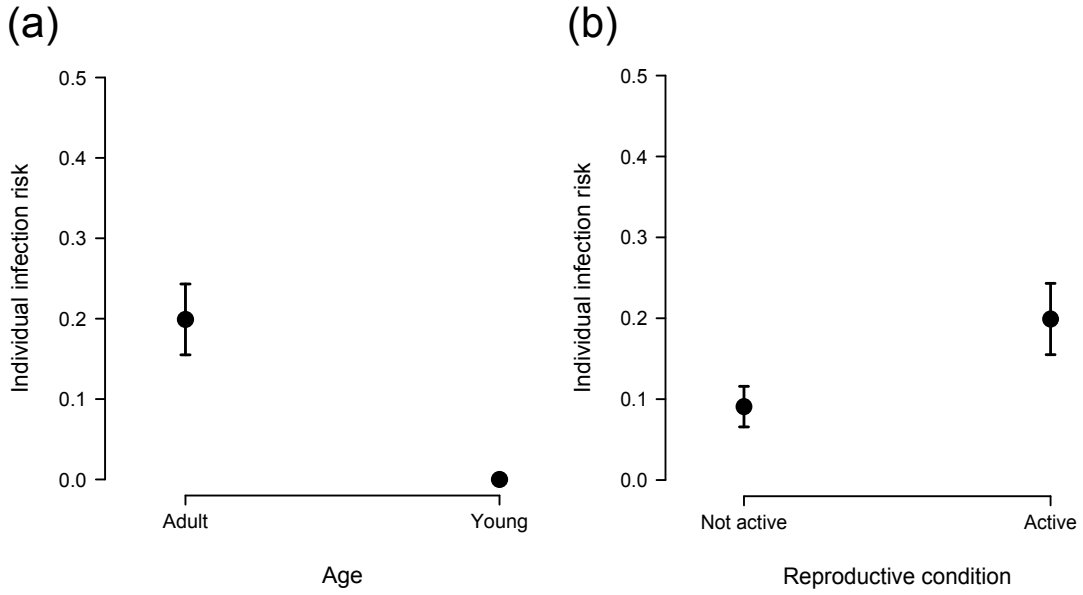

Figure 9: The predicted relationship between individual risk of *B. doshiae*-like infection in wood mice and (a) age and (b) reproductive condition. Predictions are based on the best fit model of individual infection risk. No extrinsic risk factors were identified for population level or individual level risk. Error bars indicate standard errors. Covariates and factors not being examined here are held constant as follows: age = adult, reproductive condition = active, DNA concentration = 0.6975 μg mL^{-1} (median concentration of wood mouse samples).

2.3.3.2 *B. rudakovii* (bank voles)

The best-fit model of population infection risk for *B. rudakovii* in bank voles included only *B. rudakovii* prevalence in the previous month (-0.338 ± 4.484) (Table 2.6a). No extrinsic effects were supported using the full data set, and a null model with no effects was the best-fit model in 85% of the bootstrap simulations.

The best-fit stage one model of individual infection risk included no intrinsic effects (Table 2.6b), and this model was supported in 77% of bootstrap simulations, therefore no intrinsic effects were included the second stage of modeling. The best-fit stage two model included only DNA concentration (0.008 ± 0.184). No support was found for any extrinsic effects, and a null model was identified as best fit in 71% of bootstrap simulations (Table 2.6c).

Table 2.6: Selection tables and bootstrap support for models of *B. rudakovii* infection risk in bank voles. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago. Table continued on next page.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(a) Population-level risk					
None	**2**	**43.41**	**0**	**0**	**85**
Male BV density t	3	44.98	1.56	1.56	9
#Repro inactive WM t	3	45.19	1.78	1.78	0
Female BV density t-2	3	45.24	1.83	1.83	0
#Repro active BV t	3	45.28	1.87	1.87	11
(b) Individual-level risk: Intrinsic effects					
Sex + Age	4	153.81	0	-1.86	0
Sex	3	154.00	0.19	-1.67	23
Sex + Age + Repro	5	154.85	1.05	-0.82	0
None	**2**	**155.67**	**1.86**	**0**	**77**
Age	3	155.74	1.94	0.07	0

Table 2.6: Continued from previous page.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(c) Individual-level risk: Extrinsic effects					
None	**2**	**155.67**	**0**	**0**	**71**
Year + #Repro inactive WMt-2	4	155.73	0.06	0.06	0
Male BV density t	3	156.35	0.68	0.68	16
#Repro inactive WM t	3	156.39	0.73	0.73	0
Female BV density t-2	3	156.59	0.92	0.92	0
#Repro active BV t	3	156.70	1.04	1.04	14
#Repro active BV t-2	3	156.93	1.26	1.26	0
#Repro inactive BV t	3	156.93	1.26	1.26	1
Total BV density t-2	3	157.10	1.44	1.44	0
Total BV density t	3	157.16	1.49	1.49	0
Female WM density t	3	157.17	1.50	1.50	0
#Repro inactive BV t-2	3	157.22	1.55	1.55	0
Year	3	157.28	1.61	1.61	0
Male BV density t-2	3	157.41	1.74	1.74	0
Year + Male BV density t	4	157.51	1.85	1.85	0
Male WM density t	3	157.52	1.85	1.85	0
Female BV density t	3	157.59	1.92	1.92	0

2.3.3.3 *B. grahamii* (wood mice)

There were two equally good models of population infection risk for *B. grahamii* in wood mice (Table 2.7a). One included previous month's prevalence and a positive effect of the number of reproductively active bank voles two months ago, but this model received low bootstrap support (19%). The other received substantial bootstrap support (70%) and included previous month's prevalence (-2.473 ± 4.030) and a positive effect of male bank vole density two months ago (0.053 ± 0.034).

No intrinsic effects were supported in the best fit stage one model of individual infection risk using the full data set or bootstrap simulations (Table 2.7b), and the second stage of modeling identified two equally good best fit models, which mirrored those identified for population level risk (Table 2.7c). Again, only one model was supported by the bootstrapping procedure (supported in 82% of simulations), and included an effect of DNA concentration (0.146 ± 0.140) and a positive effect of male bank vole density two months ago (0.051 ± 0.022) (Figure 2.10).

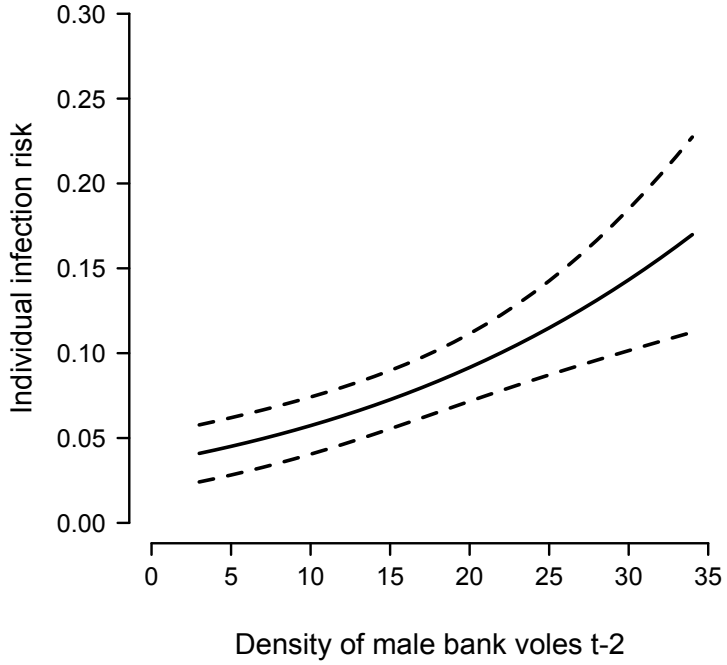

Figure 2.10: The predicted relationship between individual risk of *B. grahamii* infection in wood mice and the population density of male bank voles two months ago. Predictions are based on the single highly supported best fit model of individual infection risk. No intrinsic risk factors were identified. DNA concentration is held constant for predictions at 0.6975 μg mL^{-1} (median concentration of wood mouse samples).

Table 2.7: Selection tables and bootstrap support for models of *B. grahamii* infection risk in wood mice. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(a) Population-level risk					
#Repro active BV t-2	**3**	**47.90**	**0**	**-2.88**	**19**
Male BV density t-2	**3**	**48.57**	**0.68**	**-2.20**	**70**
Total BV density t-2	3	49.06	1.17	-1.71	22
#Repro inactive BV t-2	3	49.65	1.76	-1.12	29
None	2	50.78	2.88	0.00	29
(b) Individual-level risk: Intrinsic effects					
None	**2**	**154.4**	**0**	**0**	**100**
Age	3	155.4	1.02	1.02	0
Sex	3	155.6	1.24	1.24	0
Reproductive condition	3	156.3	1.91	1.91	0
(c) Individual-level risk: Extrinsic effects					
#Repro active BV t-2	**3**	**149.55**	**0**	**-4.80**	**19**
Year + #Repro active BV t-2	4	150.02	0.48	-4.33	1
Year + Total WM density t-2	4	150.53	0.98	-3.83	3
Male BV density t-2	**3**	**151.36**	**1.81**	**-2.99**	**82**
None	2	154.35	4.80	0	14

2.3.3.4 *B. grahamii* (bank voles)

There were three equally good models of population infection risk for *B. grahamii* in bank voles. One included previous month's prevalence and a positive effect of female wood mouse density two months ago, but received low bootstrap support (12%), whereas two others received similarly high levels of bootstrap support. The first included previous month's prevalence (-2.428 ± 0.468) and year (increased risk in 2011 compared to 2010; 1.959 ± 0.535), and was supported in 65% of bootstrap simulations; the other included previous month's prevalence (0.573 ± 1.017) and a positive effect of total wood mouse density two months ago (0.074 ± 0.020), and was supported in 58% of bootstrap simulations (Table 2.8a).

No intrinsic effects were supported in the best fit stage one model of individual infection risk using the full data or bootstrap simulations (Table 2.8b), and the second stage of modeling identified a single best fit model using the full data set (Table 2.8c). This included DNA concentration (0.007 ± 0.114) and an effect of year (increased infection risk in 2011 compared to 2010; 1.988 ± 0.540) (Figure 2.11a). However, this model was only supported in 46% of bootstrap simulations. Furthermore, another model was identified as a best-fit model in an equal number of bootstrap simulations, even though not identified when using the full data set. This model included DNA concentration (0.035 ± 0.117) and a positive effect of total wood mouse density two months ago (0.079 ± 0.020) (Figure 2.11b).

Table 2.8: Selection tables and bootstrap support for models of *B. grahamii* infection risk in bank voles. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(a) Population-level risk					
Year	**3**	**60.53**	**0**	**-14.10**	**65**
Female WM density t-2	**3**	**61.03**	**0.51**	**-13.60**	**12**
Year + Female WM density t	4	61.57	1.05	-13.06	19
Total WM density t-2	**3**	**61.98**	**1.45**	**-12.65**	**58**
None	5	74.63	14.10	0	0
(b) Individual-level risk: Intrinsic effects					
None	**2**	**311.7**	**0**	**0**	**100**
Sex	3	313.3	1.7	1.7	0
Repro	3	313.7	2.0	2.0	0
Age	3	313.7	2.0	2.0	0
(c) Individual-level risk: Extrinsic effects					
Year + Female WM density t	4	291.5	0.0	-20.2	0
Year	**3**	**292.6**	**1.1**	**-19.1**	**46**
Year + Female WM density t-2	4	292.9	1.4	-18.8	35
Year + Female BV density t	4	293.2	1.7	-18.5	0
Year + Total WM density t-2	4	293.4	1.8	-18.3	0
Year + #Repro inactive WM t-2	4	293.4	1.9	-18.3	0
None	2	311.7	20.2	0.0	1
Total WM density t-2	3				46

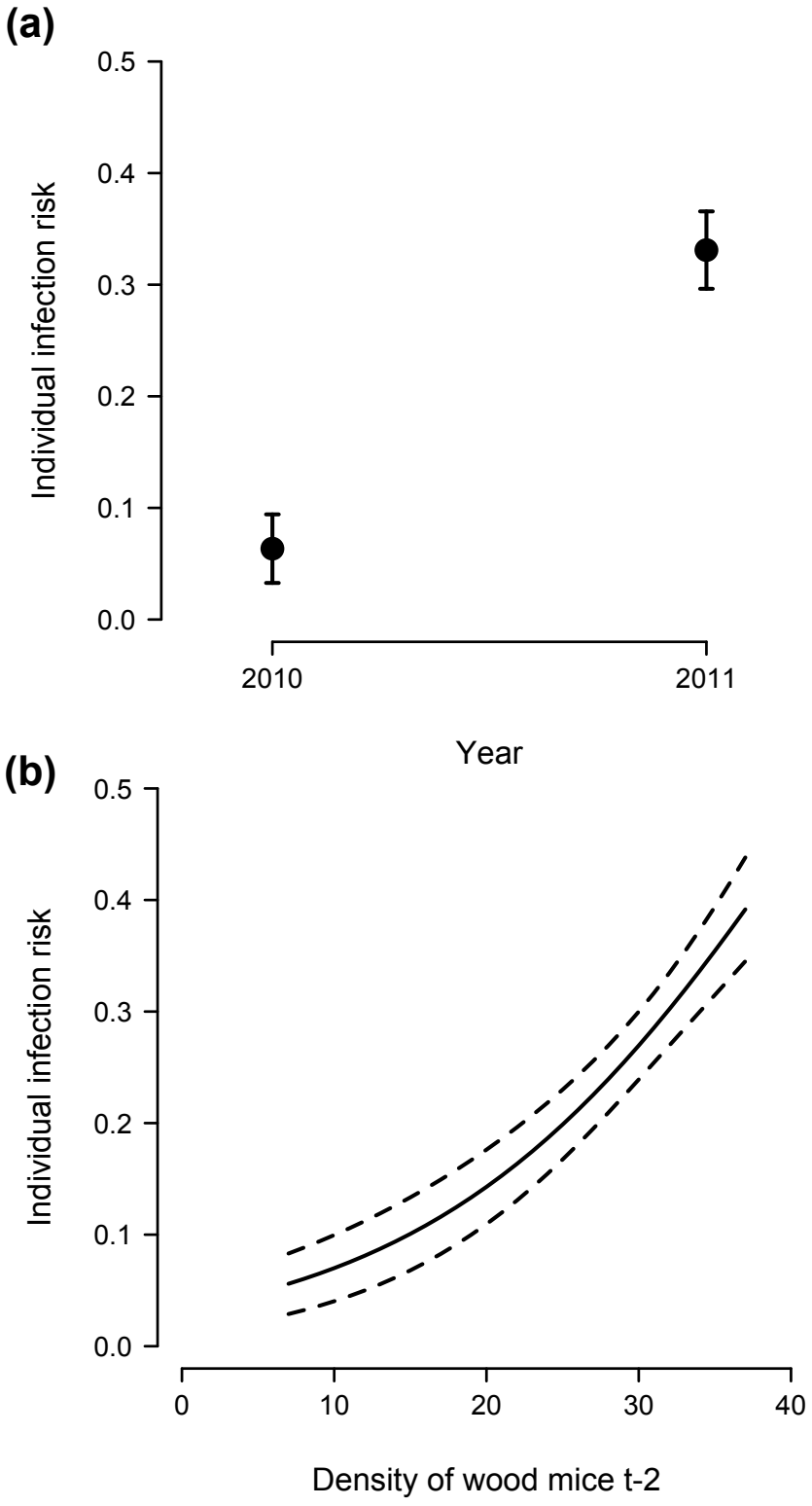

Figure 2.11: The predicted relationship between individual risk of *B. grahamii* infection in bank voles and (a) sampling year and (b) the density of wood mice two months ago. Predictions are based on two equally supported models of individual infection risk. No intrinsic risk factors were identified in either case. DNA concentration is held constant for predictions at 0.773 μg mL^{-1} (median concentration of bank vole samples).

2.3.3.5 *B. taylorii* (wood mice)

There were two equally good models of population infection risk for *B. taylorii* in wood mice (Table 2.9a). One included previous month's prevalence and effects of seasonality and year, but had low bootstrap support (17%). The other received higher bootstrap support (51%) and included previous month's prevalence (-3.553 ± 1.313), an effect of seasonality (summer peak) and a negative effect of total wood mouse density two months ago (-0.120 ± 0.028).

The best-fit stage one model of individual infection risk included an effect of reproductive condition (Table 2.9b). This model was highly supported by the bootstrapping procedure (91% of simulations identified it as a best fit model), and so this intrinsic term was incorporated into the second stage of modeling. There were three equally good stage two models (Table 2.9c), though only one was well supported by the bootstrap simulations (58%). This model did not include the effect of reproductive condition identified in stage one, and instead mirrored the best fit model for population infection risk and included DNA concentration (0.084 ± 0.124), an effect of seasonality (summer peak) and a negative effect of total wood mouse density two months ago (-0.066 ± 0.019) (Figure 2.12a-b).

Table 2.9: Selection tables and bootstrap support for models of *B. taylorii* infection risk in wood mice. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago. Table continued on next page.

Model	np	AICc	ΔAICc	ΔAICc from null	ΔAICc from seasonality	% bootstrap support
(a) Population-level risk						
Seasonality + Year	**4**	**65.67**	**0**	**-33.57**	**-16.25**	**17**
Seasonality + Total WM density t-2	**4**	**66.87**	**1.21**	**-32.36**	**-15.04**	**51**
Seasonality + Year + Total WM density t-2	5	67.65	1.98	-31.59	-14.26	0
Seasonality	3	81.92	16.25	-17.32	0	6
None	2	99.24	33.57	0	+17.32	0
(b) Individual-level risk: Intrinsic effects						
Reproductive condition	**3**	**314.2**	**0**	**-5.2**	**-**	**91**
Sex + Reproductive condition	4	315.0	0.71	-4.4	-	0
Sex*Reproductive condition	5	315.7	1.50	-3.7	-	0
Age + Reproductive condition	4	315.9	1.71	-3.5	-	0
None	2	319.4	5.19	0	-	9

Table 2.9: Continued from previous page.

Model	np	AICc	ΔAICc	ΔAICc from null	ΔAICc from seasonality	% bootstrap support
(c) Individual-level risk: Extrinsic effects						
(Intrinsic terms: Reproductive condition)						
Repro + Seasonality + Year	6	280.2	0.0	-39.2	-	0
Repro + Seasonality + Total WM density t-2	6	280.6	0.3	-38.8	-	13
Repro + Seasonality + Year + Female WM density t-2	7	280.6	0.4	-38.8	-	13
Repro + Seasonality + Year + #Repro active BV t	7	280.8	0.5	-38.6	-	0
Repro + Seasonality + Female WM density t-2	6	280.8	0.6	-38.6	-	0
Repro + Seasonality + Year + #Repro active WM t	7	281.0	0.8	-38.4	-	0
Repro + Seasonality + Year + Total WM density t-2	7	281.3	1.0	-38.1	-	0
Repro + Seasonality + Year + #Repro active WM t-2	7	281.4	1.1	-38.0	-	0
Repro + Seasonality + Year + #Repro inactive BV t	7	281.6	1.4	-37.8	-	0
Repro + Seasonality + Year + #Repro inactive WM t	7	281.6	1.4	-37.8	-	0
Repro + Seasonality + Year + Total BV density t	7	281.6	1.4	-37.8	-	0
Seasonality + Total WM density t-2	**5**	**281.7**	**1.5**	**-37.7**	**-**	**58**
Repro + Seasonality + Year + Male BV density t	7	281.7	1.5	-37.7	-	0
Repro + Year + #Repro active BV t	**5**	**281.8**	**1.5**	**-37.6**	**-**	**12**
Repro + Seasonality + Year + #Repro active BV t-2	7	281.9	1.6	-37.5	-	0
Seasonality + Year	**5**	**281.9**	**1.7**	**-37.5**	**-**	**24**
Repro + Seasonality + Year + Male WM density t-2	7	282.0	1.8	-37.4	-	0
Repro + Seasonality + Year + Male WM density t	7	282.0	1.8	-37.4	-	0
Repro + Seasonality + Year + Male BV density t-2	7	282.1	1.8	-37.3	-	0
Seasonality + Year + #Repro active WM t	6	282.1	1.9	-37.3	-	0
Repro + Seasonality + Year + Female BV density t	7	282.2	1.9	-37.2	-	0
Repro + Seasonality + Year + #Repro inactive WM t-2	7	282.2	2.0	-37.2	-	0
Repro + Seasonality + Year + #Repro inactive BV t-2	7	282.2	2.0	-37.2	-	0
None	2	319.4	37.7	0.0	-	0

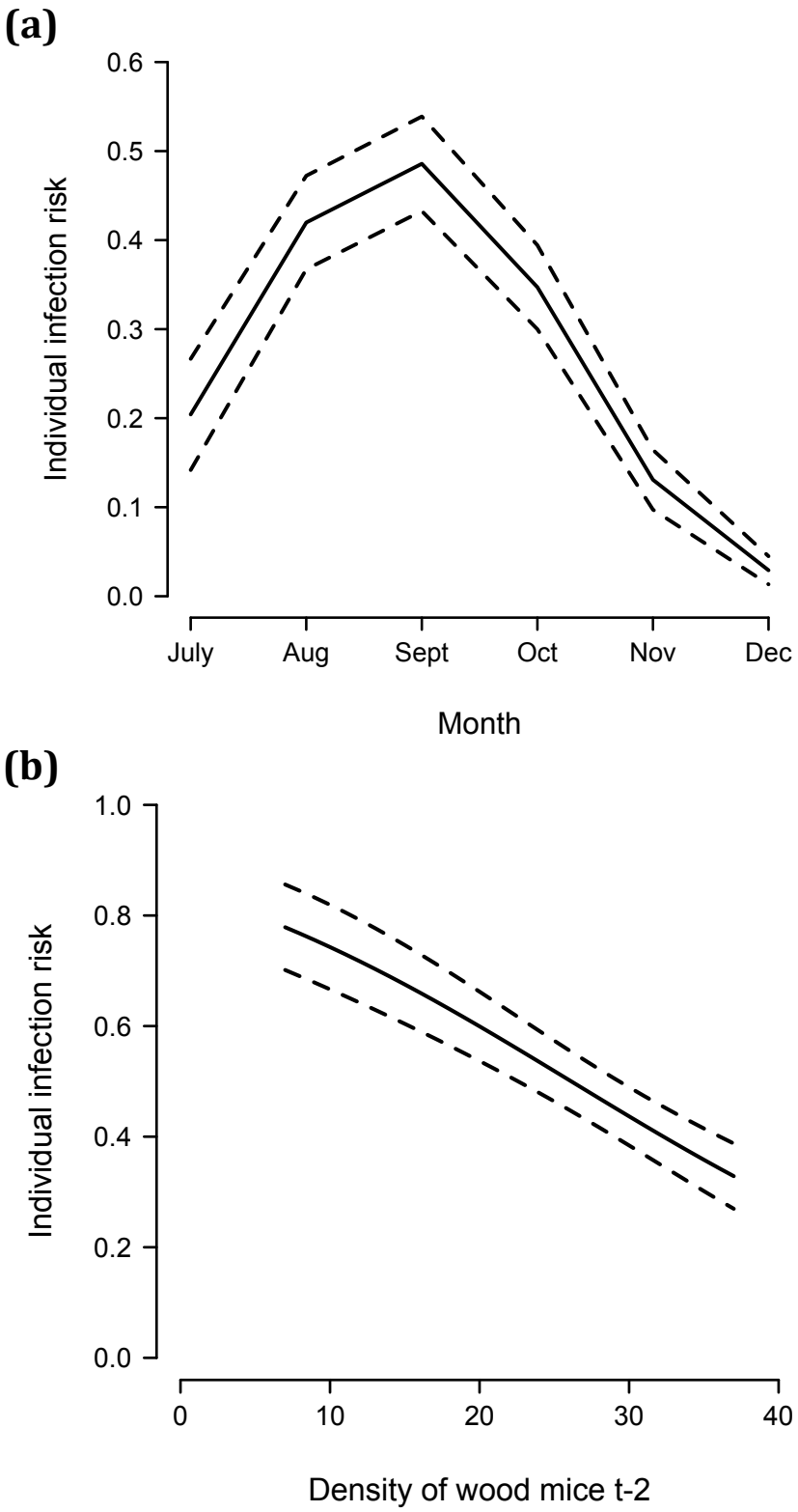

Figure 2.12: The predicted relationship between individual risk of *B. taylorii* infection in wood mice and (a) sampling month and (b) the density of wood mice two months ago. Predictions are based on a single best fit model of individual infection risk. No intrinsic risk factors were identified. Covariates and factors not being examined here are held constant as follows: wood mouse density t-2 = 27 (median), DNA concentration = 0.6975 µg mL^{-1} (median concentration of wood mouse samples).

2.3.3.6 *B. taylorii* (bank voles)

The best-fit model of population infection risk for *B. taylorii* in bank voles included only prevalence in the previous month (1.610 ± 3.821). There was no support for any extrinsic drivers of risk, and this was supported in 100% of bootstrap simulations (Table 2. 10a).

The best-fit stage one model of individual infection risk included effects of age and sex (Table 2.10b). This model was only identified as a best-fit model in 40% of bootstrap simulations. However, bootstrap support was high for a model including just an effect of sex (51%), and an effect of age was included in best-fit models of 48% of bootstrap simulations (either as a single effect, or along with sex). Both of these intrinsic drivers of risk were therefore incorporated into the second stage of modeling. The best-fit stage two model included only intrinsic effects (increased infection risk in adults compared to young individuals [2.112 ± 1.035; Figure 2.13a] and increased infection risk in males compared to females [1.035 ± 0.465; Figure 2.13b]), along with DNA concentration (-0.374 ± 0.284), and was supported in 53% of bootstrap simulations (Table 2.10c).

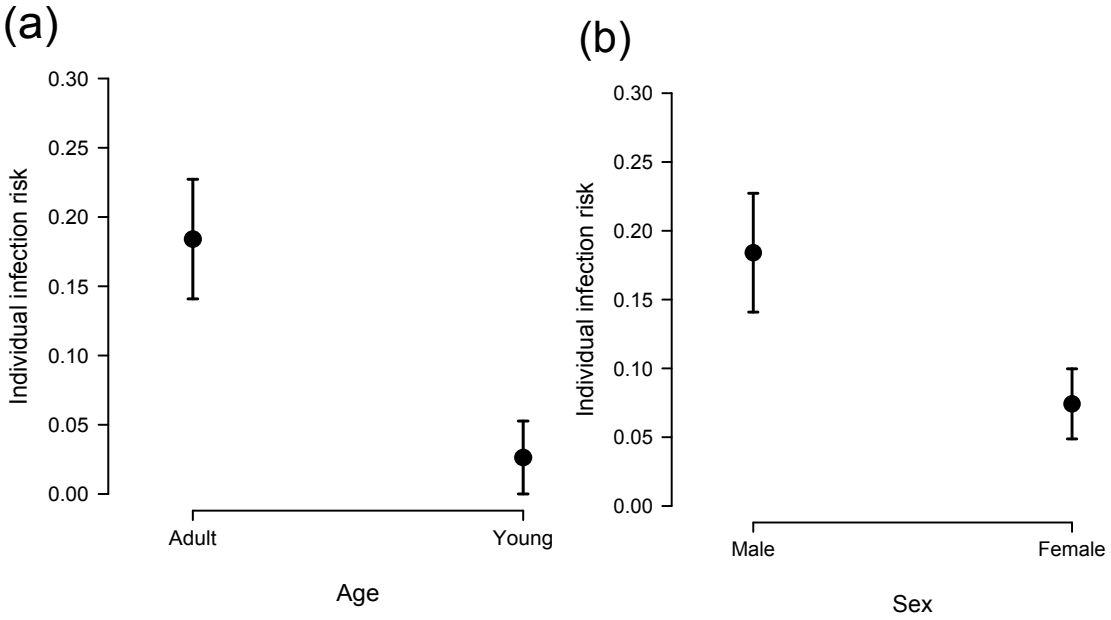

Figure 2.13: The predicted relationship between individual risk of *B. taylorii* infection in bank voles and (a) age and (b) sex. Predictions are based on a single best fit model of individual infection risk. No extrinsic risk factors were identified. Covariates and factors not being examined here are held constant as follows: age = adult, sex = male, DNA concentration = 0.773 μg mL^{-1} (median concentration of bank vole samples).

Table 2.10: Selection tables and bootstrap support for models of *B. taylorii* infection risk in bank voles. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago. Table continued on next page.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(a) Population-level risk					
None	**2**	**43.09**	**0**	**0**	**100**
Female BV density t-2	3	44.43	1.34	1.34	0
#Repro inactive BV t-2	3	44.97	1.88	1.88	0
Year	3	45.06	1.98	1.98	0
(b) Individual-level risk: Intrinsic effects					
Age + Sex	**4**	**154.5**	**0**	**-9.3**	**40**
Age + Sex*Repro	6	156.3	1.8	-7.5	0
Age + Sex + Repro	5	156.4	1.9	-7.4	0
None	2	163.8	9.3	0	0
Sex	3				51†

† Support for a single model containing both Age and Sex effects was weak, but support for an effect of Sex alone was marginally supported. Combined support for an effect of Age was also relatively high (48% of simulations found best fit models to include age alone, or age and sex), therefore both of these terms were carried through to Stage 2 models.

Table 2.10: Continued from previous page.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(c) Individual-level risk: Extrinsic effects					
(Intrinsic terms: Age + Sex)					
Age + Sex + Year + #Repro active WM t-2	6	154.2	0.0	-9.6	0
Age + Sex + #Repro inactive BV t-2	5	154.3	0.1	-9.5	0
Age + Sex + Female BV density t-2	5	154.4	0.2	-9.4	0
Age + Sex	**4**	**154.5**	**0.3**	**-9.3**	**53**
Age + Sex + Total BV density t-2	5	155.0	0.8	-8.8	0
Age + Sex + Year	5	155.4	1.2	-8.4	0
Age + Sex + Male WM density t-2	5	155.4	1.2	-8.4	0
Age + Sex + Male BV density t-2	5	155.4	1.2	-8.4	0
Age + Sex + #Repro inactive WM t-2	5	155.6	1.4	-8.2	0
Age + Sex + Seasonality + #Repro inactive BV t-2	7	155.6	1.4	-8.2	0
Age + Sex + Seasonality + #Repro inactive WM t-2	7	155.6	1.4	-8.2	0
Age + Sex + Total WM density t-2	5	155.7	1.5	-8.1	0
Age + Seasonality + #Repro inactive BV t-2	6	155.8	1.6	-8.0	0
Age + Sex + Year + #Repro inactive BV t	6	155.8	1.7	-8.0	0
Age + Sex + Year + #Repro active BV t-2	6	156.0	1.8	-7.8	0
Age + Sex + #Repro active WM t-2	5	156.0	1.9	-7.8	0
Age + Sex + Seasonality	6	156.1	1.9	-7.7	0
None	2	163.8	9.6	0	0

2.3.3.7 *B. birtlesii* (wood mice)

The best-fit model of population infection risk for *B. birtlesii* in wood mice included only prevalence the month before (0.731 ± 2.141). There was no support for any extrinsic drivers of risk, and this was supported in 62% of bootstrap simulations (Table 2.11a).

No intrinsic effects were supported in the best-fit stage one model of individual infection risk using the full data set or bootstrap simulations (Table 2.11b). The second stage of modeling identified a single best-fit model, which included DNA concentration, an effect of seasonality and a positive effect of the current density of male wood mice. However, this model was supported by none of the bootstrap simulations, and highest bootstrap support (43%) was afforded to a null model, which included only DNA concentration (0.053 ± 0.123) (Table 2.11c).

Table 2.11: Selection tables and bootstrap support for models of *B. birtlesii* infection risk in wood mice. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(a) Population-level risk					
Male WM density t	3	59.60	0	-1.15	0
#Repro inactive BV t-2	3	59.99	0.39	-0.76	24
#Repro inactive WM t	3	60.30	0.71	-0.45	11
None	**2**	**60.75**	**1.15**	**0**	**62**
Male BV density t-2	3	61.57	1.97	0.82	14
(b) Individual-level risk: Intrinsic effects					
None	**2**	**224.3**	**0**	**0**	**94**
Reproductive condition	3	224.9	0.57	0.57	6
Age	3	225.3	0.97	0.97	4
Sex	3	226.3	1.96	1.96	0
(c) Individual-level risk: Extrinsic effects					
Seasonality + Male WM density t	**5**	**219.6**	**0**	**-4.7**	**0**
Seasonality + Year + Male WM density t	6	220.9	1.3	-3.4	0
None	2	224.3	4.7	0	43
Seasonality	4	224.6	5.0	+0.3	15

2.3.3.7 *B. birtlesii* (bank voles)

The best-fit model of population infection risk for *B. birtlesii* in bank voles included only prevalence the month before (0.731 ± 2.141). There was no support for any extrinsic drivers of risk, and this was supported in 63% of bootstrap simulations (Table 2.12a).

The best-fit stage one model of individual infection risk included an interaction between sex and reproductive condition (Table 2.12b). This model was supported in 75% of bootstrap simulations, and this interaction term was therefore included in the second stage of modeling. The best-fit stage two model did not support any extrinsic drivers of infection risk (Table 2.12c). Instead, it included only the interaction between intrinsic terms previously identified (increased infection risk in females compared to males, but only when reproductively active; 2.177 ± 0.699; Figure 2.14), alongside DNA concentration (0.065 ± 0.116). While this model was only identified as a best-fit model in 31% of bootstrap simulations, the three models with strongest bootstrap support also included this term (Table 2.12c).

Table 2.12: Selection tables and bootstrap support for models of *B. birtlesii* infection risk in bank voles. **(a)** Models of population-level risk (all models include prevalence of focal *Bartonella* species in focal host species at t_{-1}) **(b)** Models of intrinsic effects on individual-level risk (all models include DNA concentration) **(c)** Models of extrinsic effects of individual-level risk. ΔAICc = difference in AICc between this model and the model with lowest AICc. All models with ΔAICc ≤ 2 are shown. Information of a null model with no intrinsic or extrinsic terms is given even if ΔAICc > 2. Best fit model(s) is in bold. Models substantiated by the bootstrapping procedure are highlighted in blue. A model is shown in grey text if it was not supported by the full data set, but received strong bootstrap support. 'np' = number of model parameters, BV = bank voles, WM = wood mice, t = current, t_{-2} = 2 months ago.

Model	np	AICc	ΔAICc	ΔAICc from null	% bootstrap support
(a) Population-level risk					
Female WM density t	3	67.70	0	-0.47	0
None	**2**	**68.17**	**0.47**	**0**	**63**
#Repro inactive BV t	3	68.83	1.13	0.66	1
Male BV density t-2	3	68.95	1.25	0.78	9
Year + Female WM density t-2	4	69.43	1.73	1.26	0
Year + Male BV density t-2	4	69.58	1.88	1.41	27
(b) Individual-level risk: Intrinsic effects					
Sex*Repro	**5**	**280.3**	**0**	**-6.4**	**75**
Age + Sex*Repro	6	281.1	0.8	-5.6	6
None	2	286.7	6.4	0	3
(c) Individual-level risk: Extrinsic effects					
(Intrinsic terms: Sex*Reproductive condition)					
Sex*Repro + #Repro inactive BV t	6	278.6	0.0	-8.1	4
Sex*Repro + Year + Male BV density t-2	7	279.4	0.7	-7.3	24
Sex*Repro + Male BV density t	6	279.7	1.1	-7.0	2
Sex*Repro + Male BV density t-2	6	279.7	1.1	-7.0	19
Sex*Repro	**5**	**280.3**	**1.7**	**-6.4**	**31†**
Sex*Repro + Seasonality	8	280.4	1.8	-6.3	0
Sex*Repro + Total BV density t	6	280.5	1.9	-6.2	0
Sex*Repro + #Repro active BV t	6	280.6	1.9	-6.1	3
None	0	286.7	8.1	0.0	0

† Bootstrap support for a model including just an interaction between Sex and Reproductive condition did not exceed 50%, but this term was included in all three models with strongest bootstrap support.

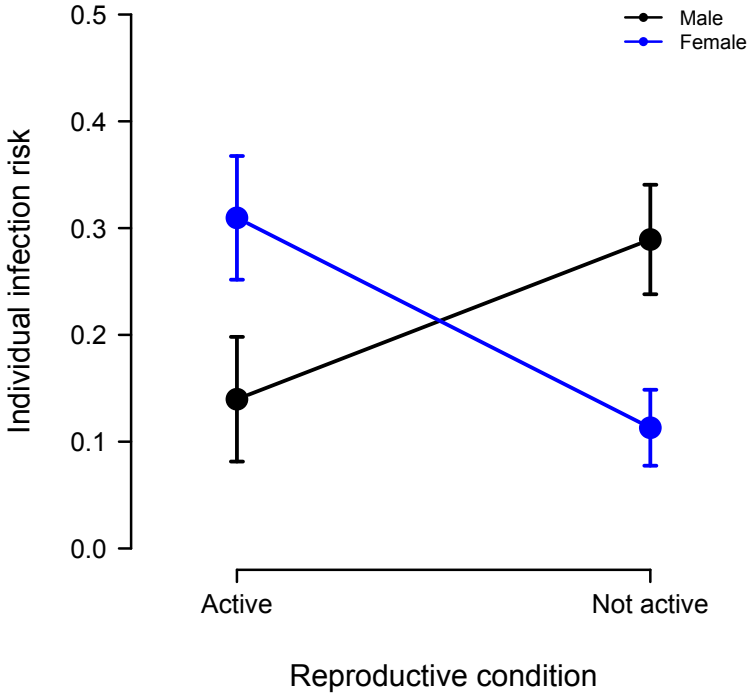

Figure 14: The predicted relationship between individual risk of *B. birtlesii* infection in bank voles and reproductive condition of males (black line) and females (blue line). Predictions are based on a single best fit model of individual infection risk. No extrinsic risk factors were identified. DNA concentration is held constant for predictions at 0.773 µg mL^{-1} (median concentration of bank vole samples).

2.4 Discussion

Bartonella parasites were common among sympatric populations of wood mice and bank voles in this study, with approximately 60% of all individuals being infected at least once. Three species of *Bartonella* (*B. grahamii*, *B. taylorii* and *B. birtlesii*) were found to infect both wood mice and bank voles, in accordance with previous studies (Birtles *et al*., 2001; Telfer *et al*., 2007a). Of these, only *B. taylorii* was consistently more prevalent in one host (wood mice) than the other. Host associations of *B. grahamii* and *B. birtlesii* were less clear; relative host prevalence for both *Bartonella* species varied across years and trapping grids, although prevalence of both parasites was higher in bank voles than wood mice for the majority of the study. These patterns of prevalence across host species are similar to those found in an earlier study at the same site (Telfer *et al*., 2007a). Other species of *Bartonella* were only found in one host species: *B. doshiae* and *B. rudakovii* in bank voles, and *B. doshiae*-like and BGA in wood mice. Determining the identity of these species required sequencing analysis, which is discussed in a later chapter (Chapter 3).

Effects of host density on risk of *Bartonella* infection were sought to infer the identity of key transmission host species for each *Bartonella* species. Models of *Bartonella* infection risk found support for the same extrinsic risk factors at both the population- and individual-levels; after accounting for seasonal and between-year variation, host population densities were evidenced as risk factors for *B. grahamii* infection in both wood mice and bank voles and for *B. taylorii* in wood mice. Specifically, risk of *B. grahamii* infection in wood mice was positively associated with the density of bank voles two months previously, therefore implicating bank voles as a key transmission host for this parasite. However, the effect was only apparent when male bank voles alone were considered, suggesting that transmission potential among different demographic groups within the bank vole population is heterogeneous, and should be considered when trying to identify key transmission hosts to. This may explain why a similar density effect was not found in an earlier study that only considered total population densities (Telfer *et al*., 2007a).

Male-biased transmission of *B. grahamii* may arise via two mechanisms. First, males may disproportionately produce the majority of infectious fleas, either because they

sustain a greater proportion of the flea population or because they are more likely to infect fleas that feed on them with *B. grahamii*. Feeding performance and reproduction of certain flea species has been found to be more successful on male hosts compared to females (Khokhlova *et al*., 2009). Furthermore, male-biased ectoparasite infestation in rodents is well documented (e.g. Krasnov *et al*., 2012; Perkins *et al*., 2003; Smith *et al*., 2005; Telfer *et al*., 2005), as is male-biased parasite infection in general (Poulin, 1996). This may be due to behavioural differences between the sexes that increase male encounter rate with parasites (Krasnov *et al*., 2005) or differences in immunocompetence that render males more susceptible to infestation/infection than females (Zuk & McKean, 1996). Secondly, in the absence of sex-biased production of infectious fleas, males may still drive infection risk by increasing the rate of contact between susceptible individuals and these infectious agents. Male bank voles have a wider home range than females (Kikkawa, 1964), who are territory holders (Flowerdew, 1993). They may therefore experience a greater rate of contact with, and opportunity to transmit infection to, a greater proportion of the population – a mechanism that has been suggested to explain the greater transmission potential of male white-footed mice (*Peromyscus leucopus*) in a rodent–helminth system in the USA where sex-associated physiological differences in parasite shedding rate were not apparent (Grear *et al*., 2012).

The possible identification of bank voles as the key transmission host for *B. grahamii* was not equivocal, as risk of *B. grahamii* infection in bank voles was not associated with bank vole density at all. Instead, infection risk was positively associated with lagged wood mouse density, contrasting with the previous result of infection risk in wood mice (and that of Telfer *et al*. (2007a), who found no support for effects of host density in the risk of *B. grahamii* infection in bank voles). However, it is important to note that a model including just an effect of between-year variation was equally supported here: risk was greater in 2011 compared to 2010. Densities of wood mice were generally higher in 2011 compared to 2010; therefore these two competing risk factors (year and wood mouse density) are correlated, which introduces problems for model selection (Graham, 2003). It is not possible to attribute variation in bank voles' risk of infection to changes in wood mouse density with any degree of certainty, as it may instead reflect subtle differences across years in environmental conditions that affect the transmissibility of *Bartonella* (e.g. between-year variation in average air temperature or relative humidity may have affected fleas development and increased the size of the vector population in 2011; Krasnov *et al*., 2001). Regardless of this, there is

no evidence that *B. grahamii* infection risk in wood mice and bank voles was associated with the density of the same rodent species; therefore it is difficult to attribute the role of key transmission host to either species.

Risk of *B. taylorii* infection in wood mice was negatively associated with lagged wood mouse density, indicating that high previous host densities reduced risk of infection. This may have arisen if previous host density exceeded the number of infectious fleas in the environment, possibly due to a high proportion of non-resident individuals within the rodent population. Such individuals may not contribute to the size of the flea population, because they do not have a nest and so do not constitute a major resource for fleas, yet still be able to pick up fleas and *B. taylorii* infection as they move transiently through the population (Krasnov *et al*., 2002). However, this mechanism requires that an infectious flea only takes a blood meal from, and infects, a single host individual, which seems unlikely. Alternatively, a previously high host density may be coupled with subsequent migration of individuals out of the population as a response to reduced resources (Anderson *et al*., 2010). If individuals infected with *B. taylorii* are less able to hold their territory, infected individuals may be more likely to leave the population. Therefore subsequent risk of infection within the remaining population may appear reduced. Evidence suggests that the prevalence of clinical manifestations due to *Bartonella* infections in is low (Schulein *et al*., 2001; Birtles, 2005), but strains of *Bartonella* from wild rodents have been found to reduce the reproductive capacity of laboratory mice (Boulouis *et al*., 2001), suggesting that infected individuals may indeed be somehow compromised. However, if either of these mechanisms were true, I would expect the same negative effect of wood mouse density to be found for infection risk of all *Bartonella* species, and it is not.

It should be noted that the best-fit model of *B. taylorii* infection risk in wood mice also included a strong seasonal component, with infection risk rising from May to September, and then falling during the autumn and winter. While this seasonality may be attributed to climatic fluctuations that impact on the development of flea vectors (Krasnov *et al*., 2005; Gage *et al*., 2008), it also mirrors the pronounced population dynamics of the wood mice. Seasonal variation in risk may itself, therefore, reflect a positive association with wood mouse density. As a result of this collinearity, the resulting negative effect of lagged wood mouse density may therefore only be capturing

residual variation of infection risk, and have little biological meaning (Mac Nally, 2000).

Importantly, there was no evidence of any association (either positive or negative) between wood mouse population density and *B. taylorii* infection risk in bank voles, indicating that whatever mechanisms underlie wood mouse infection risk are not upheld for bank voles. Indeed, there was no evidence that the density of either host species affected risk of *B. taylorii* in bank voles; therefore identification of a key transmission host for *B. taylorii* in this community cannot be resolved.

The densities of neither rodent species contributed to the infection risk of *B. doshiae*-like or *B. birtlesii* in wood mice or *B. birtlesii* or *B. rudakovii* in bank voles. Key transmission hosts could therefore not be identified here. One reason for this general lack of support for host density-associated infection risk, and indeed for the inconclusive results associated with *B. grahamii* and *B. taylorii* infection risk, is that risk of infection with a vector-borne parasite is determined by both the abundance of vectors and the rate at which they become infectious. Within a multi-host community, different species may affect these processes to different degrees, resulting in complex drivers of infection risk that have been widely discussed (e.g. LoGuidice *et al*., 2003; Diaz *et al*., 2006; Randolph & Dobson, 2012; Wood & Lafferty, 2013). An overriding single key transmission host may not be apparent for the *Bartonella* parasites in this study system, because fleas may become infectious carriers of a *Bartonella* species by feeding primarily on a particularly "competent" host species, but both wood mice and bank voles (and perhaps other rare, unsampled rodent species) may determine the overall abundance of fleas within the community. The relative role of a particular host species in determining infection risk may therefore only be apparent when considering the overall species composition of the community. Species composition here was not related in any consistent way to absolute host densities, due to variation between the relative population dynamics of wood mice and bank voles across trapping grids and years. Assessing the role of community composition alongside host densities is therefore difficult. Furthermore, the combined effects of wood mouse and bank vole densities could not be investigated within the same statistical models due to their high degree of correlation.

Interestingly, accounting for potential intrinsic variation related to age, sex and reproductive status within models of individual infection risk did not alter the support that emerged for the extrinsic effects investigated, indicating that inherent variation in the susceptibility of individuals did not confound the extrinsic effects identified in models of population infection risk. In fact, support for intrinsic risk factors was only found in cases where extrinsic risk factors were not supported, indicating that risk of infection is largely determined by individuals' external environment rather than their own susceptibility. A study of *Bartonella* infection in populations of wood mice in Ireland also showed that infection risk was related more to extrinsic risk factors than an individual's age, sex or reproductive maturity (Telfer *et al*., 2005).

However, intrinsic risk factors were identified for some *Bartonella* species here. Older individuals had an increased risk of infection than younger individuals in some cases (*B. doshiae*-like in wood mice and *B. taylorii* in bank voles). This may be because young individuals harbour protective maternal antibodies, although another study found that individuals were in fact more likely to be infected with *Bartonella* spp. when young (Paziewska *et al*., 2012), and vertical transmission has been demonstrated in cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) (Kosoy *et al*., 1998). Alternatively, increased exposure over time may result in the higher risk of infection in adults seen here, and in another previous study of *Bartonella* infections in field voles (Telfer *et al*., 2007b).

Reproductively active individuals were also at greater risk of infection than reproductively inactive individuals in some cases (*B. doshiae*-like in wood mice), which may result from higher rates of exposure due to more frequent contacts with other individuals and their fleas during mating (Marshall, 1981). Interestingly, risk of *B. birtlesii* infection in bank voles was higher in females when they were reproductively active, but lower in males, which contradicts an explanation based solely on increased susceptibility to parasite infection due to increased testosterone levels in reproductively active males (Zuk & McKean, 1996). However, male bank voles in general (reproductively active or not) experienced greater risk of *B. taylorii* infection compared to females, which may be explained by differences in risk-related behaviour or physiological differences that render males more susceptible to infection than females (Zuk & McKean, 1996). Interestingly, I did not find support for consistent intrinsic effects on infection risk across *Bartonella* species. If intrinsic effects were driven by

differences in risk-related behaviours between demographic groups, I would expect to find support for the same intrinsic effects across *Bartonella* species. This inconsistency suggests that physiological differences across demographic groups are more key in driving these emergent pattern of intrinsic risk, and that these differences impact risk to varying degrees across *Bartonella* species.

It should be noted that an ability to detect any host density effects on *Bartonella* infection risk might have been limited by the short length of the time series available. Indeed, the effects of host density on infection risk in several other studies have only been detected with time lags of at least three months (e.g. *Bartonella* in wood mice and bank voles, Telfer *et al*., 2007a; cowpox virus in field voles, Burthe *et al*., 2006). However, given that rodent fleas develop from eggs to haematophagous adults within ~4-6 weeks under suitable conditions (Marshall, 1981) and detectable bacteremia develops rapidly in *Bartonella*-infected individuals (approximately 4 days in experimentally infected rodents; Schülein *et al*., 2001), it is likely that any effects would be evident with the two-month lag investigated here. The short time series also constrained the investigation of infection seasonality, and there was no evidence for the basic seasonal patterns in the majority of statistical models, contrary to earlier studies of rodent *Bartonella* (Telfer *et al*., 2007a). Limitations may also be associated with measuring population densities on discrete trapping grids within an area of continuous woodland, as migration of individuals into and out of these areas was likely. The sudden appearance of young, mostly sub-adult, individuals on trapping grids before reproductively active individuals had time to reproduce indicates immigration of individuals from elsewhere. Trapping grid densities may therefore have less explanatory power than anticipated, as hosts not included in measures of population densities may have influenced the infection risk of resident individuals.

An interesting possibility that was not investigated in this study is that the identity of key transmission hosts within a multi-host community may be seasonally variable. In this system, different species of flea may be more abundant at different times of year (Whitaker, 2007). If these flea species rely on different rodent host species for reproduction, and assuming vector competency for *Bartonella* is constant across flea species, then the abundance of the prevailing flea population and associated risk of *Bartonella* infection may depend on a different host species population at different times of year. Attempting to identify a single species as the key transmission host may

therefore be unsuccessful and of little practical value. However, the nature of the relationship between rodent density and flea abundance in general is unclear, and not necessarily linear (Krasnov *et al*., 2002; Stanko *et al*., 2002; Telfer *et al*., 2007b). Indeed, the effect of host density on flea abundance has been found to vary seasonally and be related to specific age groups even with respect to a single host species (field voles, *Microtus agrestis*; Smith *et al*., 2005). Identifying seasonal variation in key host identity would therefore be an exciting but challenging task.

Finally, it is possible that while wood mice and bank voles are broadly sympatric in their distributions, they may be infested by different species of flea, meaning that one host species is unlikely to affect risk of *Bartonella* infection in the other. However, several of the same flea species have been found to infect sympatric wood mice and bank voles in the UK (Noyes *et al*., 2002) and Ireland (Telfer *et al*., 2005), and other sympatric host species in rodent communities elsewhere in Europe (Harris *et al*., 2009), indicating that specific relationships between fleas and hosts do not exist. However, subtle differences in patterns of daily activity (Greenwood, 1978) and microhabitat preferences (Bergstedt, 1965; Geuse, 1985) may limit the transfer of infectious fleas between host species, even if the same flea species infest both. As a result, flea populations and therefore some or all of the apparently shared *Bartonella* parasites identified here may circulate independently within wood mouse and bank vole populations, which may explain the general lack of shared host density effects identified. Independent circulation of apparently shared parasites has been demonstrated in these rodent communities before (e.g. cowpox virus; Begon *et al*., 1999). An explicit investigation of host associations with different flea species, the degree of home-range overlap between wood mice and bank voles, and finer-scale genetic characterisation of the *Bartonella* population would be valuable in identifying whether *Bartonella* parasites also circulate independently within these sympatric wood mouse and bank vole populations.

Understanding heterogeneities of parasite transmission within multi-host communities is necessary for the successful management of disease risk in nature (Haydon *et al*., 2002; Fenton & Pedersen, 2005; Streicker *et al.*, 2013). Here I have investigated these transmission heterogeneities simultaneously for several different *Bartonella* parasites within a community of rodents. Despite the inherent difficulties of investigating complex community interactions using observational data, I have identified at least one

parasite (*B. grahamii*) for which infection risk in one host species may be influenced by the dynamics of a different host species, and uncovered further potential complexities related to sex-associated differences in a host species' contribution to transmission. Further study of this rodent-*Bartonella* system, by integrating a variety of approaches (Viana *et al*., 2014) and focusing on the areas suggested above, will increase our understanding of this important multi-host-multi-parasite model, and ultimately contribute to a more general appreciation of parasite transmission within a community context.

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2.6 Appendix

Explanation of the sinusoidal seasonality term

This commonly used seasonal term (e.g. Carslake *et al*., 2005, Telfer *et al*., 2007a) implements the rules of trigonometry, such that it fits a sine wave to the data, offset on the x-axis according to the periodicity specified. It takes the following form:

$\beta_1 \sin(2*\pi*t/12) + \beta_2 \cos(2*\pi*t/12)$

where t is the numerical representation of trapping month (e.g. January=1, December=12), and the denominator in each term represents 12-month periodicity. Data here were not collected from a complete annual time period; therefore potential seasonal patterns of infection risk are not obvious. However, a 12-month cycle is the most parsimonious assumption (we have no basis on which to form an alternative hypothesis), and also reasonable when considering the results of a previous study of *Bartonella* infection risk at the same location, which incorporated an identical seasonality component into statistical models (Telfer *et al*., 2007a). Although the pattern of seasonality may vary between years, it was not possible to test for this interaction here owing to the asymmetry of the data collected (data were not collected from all sessions in both years, and not all grids were trapped in both years), which limits the power of this analysis.

Wood mouse and bank vole capture rates

Wood mice and bank voles did not differ in their rates of within-session capture per individual (range 1-3 and median=1 for both host species; Wilcoxan ranked sum test W=94903.5, p=0.84), the number of unique sessions captured per individual (range 1-6 for voles and 1-7 for mice, median=1 for both, W=30664.5, p=0.88), or the minimum number of months known alive per individual (range 1-16 and median = 1 for both species, W=28719.5, p=0.16).

While capture histories suggest little variation between host species in their longevity within the study or propensity for capture, a slightly greater percentage of wood mice were known alive within the study area for >2 sessions (30.4%) compared to bank voles (23.8%), which may indicate an increased rate of mortality or dispersal of bank voles outside of the trapping area compared to wood mice. Coupled with the fact that host species do not differ in the number of sessions in which they were captured, the greater longevity of wood mice also indicates a greater number of "missed" captures of wood mice compared to bank voles, which suggests a reduced trap affinity in wood mice compared to bank voles. Wood mice have previously been found to have a greater home range diameter than bank voles (Carslake *et al*., 2005), which may partly explain this pattern, if our trapping grids only encompass part of individuals' home ranges.

Correlated population-related variables

Population-related variables for each host species were often highly correlated with each other. This meant that additive effects of multiple population-related variables could not be investigated within the same statistical models, without over-parameterisation.

Tables below show pair-wise Spearman's rank correlation coefficients.
(NS if $p > 0.05$, $^{+}$ if $p < 0.10$, * if $p < 0.05$, ** if $p < 0.01$, *** if $p < 0.001$)

Table A2.3.1: Spearman's rank correlations between wood mouse population metrics

	Total density	**# Repro active**	**# Repro inactive**	**# Males**	**# Females**	**# Adults**
# Repro active	0.60**					
# Repro inactive	0.54**	-0.18^{NS}				
# Males	0.88***	0.53**	0.57**			
# Females	0.73***	0.34^{NS}	0.52**	0.44*		
# Adults	0.81***	0.46*	0.63**	0.89***	0.56**	
# Young	0.32^{NS}	0.37^{NS}	0.25^{NS}	0.27^{NS}	0.16^{NS}	0.11^{NS}

Table A2.3.2: Spearman's rank correlations between bank vole population metrics

	Total density	**# Repro active**	**# Repro inactive**	**# Males**	**# Females**	**# Adults**
# Repro active	0.78***					
# Repro inactive	0.87***	0.54**				
# Males	0.88***	0.71***	0.90***			
# Females	0.84***	0.76***	0.60**	0.61***		
# Adults	0.91***	0.79***	0.85***	0.84***	0.76***	
# Young	0.76***	0.51*	0.85***	0.90***	0.50*	0.65***

The population densities of wood mice and bank voles were also often correlated within the same trapping session. This meant that additive effects of host species densities could not be investigated within the same statistical models, without over-parameterisation.

Table A2.3.3: Spearman's rank correlations between host species population metrics

Total density	0.52**
# Repro active	0.65***
# Repro inactive	-0.13^{NS}
# Males	0.42*
# Females	0.45*
# Adults	0.27^{NS}
# Young	0.26^{NS}

There was also a high degree of temporal correlation within each population metric, such that metrics calculated at time points only 1 month apart were generally more highly correlated than those calculated 2 months apart. I therefore restricted the investigation of all density effects to current densities and densities lagged at t-2 months.

Table A2.3.4: Speaman's rank correlations between population metrics separated by one and two months, for each host species.

		Wood Mice		**Bank Voles**	
		t	**t-1**	**t**	**t-1**
Total density	**t-1**	0.72***		0.50*	
	t-2	0.12^{NS}	0.63*	0.55*	0.58*
Reproductively active	**t-1**	0.31^{NS}		0.52*	
	t-2	-0.03^{NS}	0.05^{NS}	0.25^{NS}	0.51*
Reproductively inactive	**t-1**	0.62**		0.36^{NS}	
	t-2	0.09^{NS}	0.57*	-0.23^{NS}	0.29^{NS}
Males	**t-1**	0.71***		0.49*	
	t-2	0.14^{NS}	0.61**	0.20^{NS}	0.44^{NS}
Females	**t-1**	0.69**		0.34^{NS}	
	t-2	0.31^{NS}	0.70**	0.18^{NS}	0.57*
Adults	**t-1**	0.55*		0.44^{+}	
	t-2	0.33^{NS}	0.43^{+}	0.25^{NS}	0.43^{+}
Young	**t-1**	0.52*		0.05^{NS}	
	t-2	-0.02^{NS}	0.42^{+}	-0.28^{NS}	0.19^{NS}

Table A2.4.1: Parameter estimates for all best-fit models of population-level infection risk. †† there were two equally good best fit models of infection risk for *B. grahamii* in bank voles. Parameter estimates for both of these models are given.

	β	S.E.	*z*	*p*
Models of infection risk in Wood Mice				
B. doshiae				
Intercept	-2.668	0.417	-6.396	<0.001
B. doshiae prevalence in WM t-1	4.208	2.334	1.803	0.07
B. grahamii				
Intercept	-3.039	0.509	-5.973	<0.001
B. grahamii prevalence in WM t-1	-2.473	4.030	-0.614	0.54
Male BV density t-2	0.053	0.034	2.295	0.02
B. taylorii				
Intercept	-0.381	0.772	-0.494	0.62
B. taylorii prevalence in WM t-1	-3.553	1.313	-2.705	0.01
cos(2πSession/12)	-4.592	1.056	-4.347	<0.001
sin(2πSession/12)	2.529	0.468	5.399	<0.001
Total WM density t-2	-0.120	0.028	-4.206	<0.001
B. birtlesii				
Intercept	-1.851	0.364	-5.092	<0.001
B. birtlesii prevalence in WM t-1	0.731	2.141	0.341	0.733
Models of infection risk in Bank Voles				
B. grahamii††				
Intercept	-2.428	0.468	-5.183	<0.001
B. grahamii prevalence in BV t-1	-0.903	1.120	-0.806	0.42
Year				
2010				
2011	1.959	0.535	3.660	<0.001
Intercept	-3.272	0.652	-5.020	<0.001
B. grahamii prevalence in BV t-1	0.573	1.017	0.564	0.57
Total WM density t-2	0.074	0.020	3.663	<0.001
B. taylorii				
Intercept	-2.480	0.471	-5.267	<0.001
B. taylorii prevalence in BV t-1	1.610	3.821	0.421	0.67
B. birtlesii				
Intercept	-1.659	0.317	-5.226	<0.001
B. birtlesii prevalence in BV t-1	2.230	1.275	1.749	0.08
B. rudakovii				
Intercept	-2.367	0.372	-6.357	<0.001
B. rudakovii prevalence in BV t-1	-0.338	4.484	-0.75	0.94

Table A2.4.2: Parameter estimates for all best-fit models of individual-level infection risk. †† there were two equally good best fit models of infection risk for *B. grahamii* in bank voles. Parameter estimates for both of these models are given.

	β	S.E.	*z*	*p*
Models of infection risk in Wood Mice				
B. doshiae				
Intercept	-1.401	0.332	-4.220	<0.001
DNA concentration	0.012	0.170	0.071	0.94
Age				
Young				
Adult	16.301	1029	0.016	0.98
Reproductive condition				
Not active				
Active	0.912	0.405	2.250	0.02
B. grahamii				
Intercept	-3.407	0.516	-6.606	<0.001
DNA concentration	0.146	0.140	1.041	0.30
Male BV density t-2	0.051	0.022	2.284	0.02
B. taylorii				
Intercept	-1.480	0.662	-2.236	0.03
DNA concentration	0.084	0.124	0.676	0.50
cos(2πSession/12)	-2.565	0.639	-4.018	<0.001
sin(2πSession/12)	1.837	0.364	5.045	<0.001
Total WM density t-2	-0.066	0.019	-3.525	<0.001
B. birtlesii				
Intercept	-1.800	0.220	-8.199	<0.001
DNA concentration	0.053	0.123	0.432	0.67
Models of infection risk in Bank Voles				
B. grahamii††				
Intercept	-2.697	0.524	-5.143	<0.001
DNA concentration	0.007	0.114	0.065	0.95
Year				
2010				
2011	1.988	0.540	3.683	<0.001
Intercept	-3.406	0.660	-5.163	<0.001
DNA concentration	0.035	0.117	0.298	0.77
Total WM density t-2	0.079	0.020	2.899	<0.001
B. taylorii				
Intercept	-2.250	0.442	-5.333	<0.001
DNA concentration	-0.374	0.284	-1.317	0.19
Age				
Young				
Adult	2.112	1.035	2.048	0.04
Sex				
Female				
Male	1.035	0.465	2.225	0.03
B. birtlesii				
Intercept	-0.845	0.291	-2.916	<0.01
DNA concentration	0.065	0.116	0.561	0.57
Sex*Reproductive condition				
Male*Not active	2.177	0.699	3.114	<0.01
B. rudakovii				
Intercept	-2.411	0.300	-8.029	<0.001
DNA concentration	0.008	0.184	0.043	0.97

Chapter 3

Covert specificity of apparently shared *Bartonella* parasites in a wild rodent community.

3.1 Introduction

Most parasites, including micro- and macro-parasites, appear to infect multiple host species in nature (Cleaveland *et al*., 2001; Woolhouse *et al*. 2001; Pedersen *et al*., 2005; Begon *et al*., 1999). Transmission between host species may have consequences for parasite persistence and determine an individual's risk of infection in natural communities (Holt *et al*., 2003; Dobson, 2004). Determining the nature of transmission between sympatric host species therefore offers the potential to target control measures that can reduce disease risk in multi-host communities (e.g. Donnelly et al. 2006; Kaare et al. 2009).

Identifying the extent to which sympatric host species transmit generalist parasites to each other, and therefore the structure of the parasite "maintenance community" (Haydon *et al*., 2002), is challenging however, as similar patterns of parasite prevalence within host populations may result from very different underlying transmission processes (Fenton & Pedersen, 2005; Viana *et al*., 2014). At one extreme, a parasite may only be maintained within a host species population due to regular spillover transmission from another "key" host species (i.e. an "apparent" multi-host generalist). For example, rabies virus infections in wild carnivores in the Serengeti are only maintained through regular transmission from domestic dogs (Lembo *et al*., 2007). At the other extreme, transmission may occur equally within and between host species, with each species contributing to parasite maintenance within the community as a whole (i.e. a "true" multi-host generalist; Fenton & Pedersen, 2005). For example, brucellosis infections in Yellowstone National Park are maintained endemically within populations of cattle, bison and elk (Dobson & Meagher, 1996). Being able to identify whether certain host species are disproportionately responsible for the majority of parasite transmission within a multi-host system is crucial for determining which individuals to target for effective disease management (Streicker *et al*., 2013).

Further complications arise from the fact that what appears to be a true multi-host parasite may actually comprise distinct subsets of the parasite population that circulate independently within sympatric host species populations (e.g. Begon *et al*., 1999). In addition, recent molecular advances have revealed a high degree of genetic diversity within individual parasite species (Poulin & Keeney, 2008), and covert host-specificity of genetic variants has been identified in a number of cases. Examples can be found across a broad range of parasite taxa, including viruses (e.g. Rabies virus: Rupprecht *et al*., 1987; Smith *et al*., 1995; Rupprecht *et al.*, 2002), bacteria (e.g. *Anaplasma phagocytophilum*: Foley *et al*., 2009; Rejmanek *et al*., 2012), protozoa (e.g. *Plasmodium* spp. infecting *Anolis* lizards in the Caribbean: Perkins *et al*., 2000; *Leucocytozoidae* spp. infecting Californian raptors: Sehgal *et al*., 2006), helminths (e.g. Trematodes infecting *Diplodus* fishes in the north-west Mediterranean: Jousson *et al*., 2000; *Neoechinorhynchus golvani* cestodes infecting Mexican fish: Martinez-Aquino *et al*., 2009), and various ectoparasites (e.g. mites infesting Galapagos bird species: Whiteman *et al*., 2006; feather lice infesting doves in the Americas: Johnson *et al*., 2002). These studies suggest that covert host-specificity of what appear to be multi-host parasites may be a widespread phenomenon, and that parasite transmission between species in multi-host communities may therefore be less common than previously thought.

When such covert host-parasite specificity is unearthed within sympatric multi-host communities, it indicates that there may be previously unidentified barriers to between-species transmission. Such barriers demand further investigation to understand their nature and how they may influence future disease emergence. Barriers to transmission may arise from ecological or physiological incompatibilities between hosts and parasites (Combes, 2001). Differences in the ecology of host species (e.g. diet or microhabitat preferences) may result in few opportunities for the transmission of a parasite between them (e.g. Jousson *et al*., 2000), eventually leading to divergence of the parasite into strains that appear to be host-specific, but would be able to infect either host species given the opportunity (ecological incompatibility). Alternatively, genetic variants of a parasite may represent functionally divergent cryptic species with the physiological capability to infect only a particular host species, even when the opportunity to use other species as a host is available (physiological incompatibility)

(e.g. Quinnell *et al*., 1991). Regardless of whether ecological or physiological incompatibilities are at play, the identification of genetically distinct host-specific variants of a parasite previously classified as a multi-host generalist would be evidence that transmission between the sympatric host species does not play a direct role in parasite maintenance within the host community, which will have implications for effective disease control.

Bartonella parasites of woodland rodents offer a useful system in which to study the genetic structure of parasites that appear to infect multiple host species. These gram-negative proteobacteria are haemoparasites of a diverse range of mammalian hosts (Breitschwerdt & Kordick, 2000; Kosoy *et al*., 2012). Several different species have been detected within wild rodents (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al*., 2007a; Knap *et al*., 2007; Bray *et al*., 2007; Gil *et al*., 2010; Welc-Faleçiak *et al.,* 2010; Paziewska *et al*, 2012), and transmission between individuals occurs chiefly via the feeding activity of haematophageous flea vectors (Bown *et al*., 2004; Morrick *et al*., 2011). Several species of *Bartonella* have been found to circulate endemically within mixed wood mouse (*Apodemus sylvaticus*) and bank vole (*Myodes glareolus*) communities in the UK, and importantly, several of the same species are reported to infect both of these rodent species in sympatry (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al.,* 2007b; Chapter 2). Previous longitudinal studies have found some evidence for host species density effects on prevalence in some cases, which may suggest possible between-host species transmission routes, but in many cases they were inconclusive (Telfer *et al*., 2007b; Chapter 2). Therefore, whether or not transmission of *Bartonella* spp. occurs between wood mice and bank voles, and the importance of such transmission for parasite maintenance in these host populations, remains unresolved.

One important consideration, and a potential reason for previous inconclusive results regarding the role of between-species transmission, is that sympatric populations of wood mice and bank voles may harbour discrete and distinct subsets of the same *Bartonella* species. Methods for parasite diagnosis in previous studies of *Bartonella* spp. in UK wood mice and bank voles (e.g. Telfer *et al*., 2007b; Chapter 2) have commonly relied on a high-throughput laboratory technique that identifies infections to species-level but is unable to detect genetic variability within *Bartonella* species (Telfer *et al*., 2005). Any associations between sub-specific parasite variants and host species

would therefore have gone undetected, and a *Bartonella* species found to infect both hosts would instead be assumed to be a multi-host generalist.

Supporting this possibility, a study of *Bartonella* infections in Irish rodents (Telfer *et al*, 2005) revealed complexities regarding *Bartonella* transmission between populations of wood mice and bank voles. Infections within recently introduced bank voles were absent, even though there was a high prevalence in sympatric wood mice of *Bartonella* species found to infect bank voles in England, suggesting that transmission from wood mice to bank voles could not occur. Among other possibilities, the authors suggested that introduced bank voles might not have been susceptible to the native *Bartonella* strains found in Ireland. In fact, bank voles may not have been susceptible to *Bartonella* strains in Ireland simply because they are not susceptible to strains that infect wood mice, and they did not bring their own host-specific strains with them. Indeed, sub-species genetic variation of *Bartonella* parasites and specific host associations has been demonstrated elsewhere in other rodent communities. Experimental infections of rodents in the USA found that bacteremia in cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) would only establish when hosts were inoculated with the same citrate synthase (*glt*A) *Bartonella* variant that was originally obtained from the same species (Kosoy *et al*., 2000). Also, in a study of rodent *Bartonella* infections in Poland, several *glt*A variants were detected, and significant associations were found between clades of *Bartonella* variants and host species according to nested clade analysis (Paziewska *et al*., 2011).

Through extensive sampling of individual hosts and the genetic characterisation of their *Bartonella* infections, the present study aimed to establish, for the first time, (1) whether sub-specific genetic variation exists within populations of *Bartonella* that infect sympatric wood mice and bank voles in the UK and (2) whether different variants of the same *Bartonella* species are associated with different host species. If true, this would suggest a limited role for between-host species transmission in maintaining some *Bartonella* variants in the rodent community, therefore questioning the classification of such species as multi-host generalists. More generally, this work aims to highlight the importance of fine-scale characterisation of parasite populations in understanding the complexities of parasite persistence within multi-host communities and identifying the most appropriate host species targets for effective disease control.

3.2 Methods

3.2.1 Sample collection

Wood mice and bank voles were monitored longitudinally within their natural woodland habitats at three sites in northwest England: Manor Wood (MW; N 53.3301°, E -3.0516°), Maresfield & Gordale woods (MFG; N 53.2729°, E -3.0615°) and Rode Hall (RH; N 53.1213°, E -2.2798°) (Figure 3.1). Sherman live-traps (Alana Ecology, UK), baited with mixed grain and carrot, were deployed in pairs at 10m intervals within discrete trapping grids at each site. Four grids were employed at MW (two 50m x 50m and two 70m x 70m), and three grids at both MFG and RH (all 50m x 50m). Trapping sessions took place every four weeks from May to December in 2011 at MW, and in 2012 at MFG and RH. Upon first capture, all individuals were fitted with a sub-cutaneous electronic passive induced transponder (PIT-tag), enabling identification of individuals when re-captured. When first captured within a monthly trapping session, a small blood sample (~30µL) was taken from the tail tip of each individual for subsequent characterisation of *Bartonella* infections, and morphometric data were taken. Further details of field methods are given in Chapter 2.

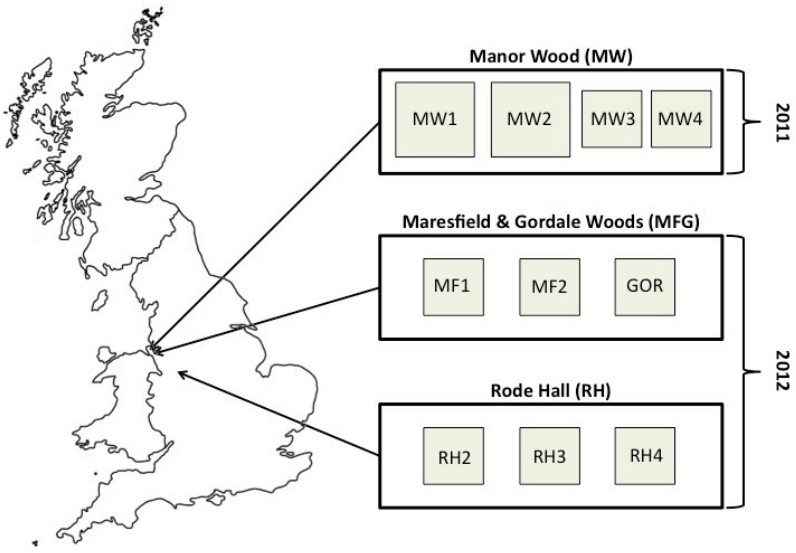

Figure 3.1: Locations of three woodland sites in northwest England in which rodents were captured during 2011 or 2012. Green squares within each woodland rectangle refer to the trapping grids employed at each site. Larger trapping grids were 70mx70m in size; smaller trapping grids were 50mx50m in size.

3.2.2 Characterisation of *Bartonella* infections

DNA was extracted from blood samples as detailed in Chapter 2 (Section 2.2.2.1). Extractions were then used as templates in semi-nested polymerase chain reactions targeting a 300-500bp fragment of the *Bartonella* 16S-23S internal transcribed spacer (ITS) region, using the genus-specific primers of Telfer *et al*. (2005) (first round primers: *bigF* and *bogR*; second round primers: *bigF* and *bigR*). Full details of primer sequences, PCR reaction mixtures and thermal cycling programmes are given in Chapter 2 (Section 2.2.2.2). Blood samples positive for *Bartonella* infection were identified by the presence of a PCR amplicon when run on a 2% (w/v) agarose gel, stained with Ethidium Bromide, and run for 30 minutes at 120V.

3.2.2.1 Characterisation according to the length of the partial 16S-23S ITS region

Bartonella infections were characterised in two ways. First, where samples were found to be positive, all infections were categorised into one of five groups (A-E) based on PCR amplicon size (following the methods of Telfer *et al*., 2005, Telfer *et al*., 2007, and detailed in Chapter 2, Section 2.2.2.2) (Figure 3.2). The length of the partial 16S-23S ITS (pITS) region targeted here is hypervariable between different *Bartonella* species (Roux & Raoult, 1995; Birtles *et al*., 2000; Houpikian & Raoult, 2001); Therefore amplicon size categories broadly correspond to species-level identifications, providing a useful, high-throughput method of diagnosis (Table 3.1). Co-infection with more than one *Bartonella* species may also be revealed using this method, by the presence of multiple amplicons of different size. The proportions of wood mice and bank voles positive at least once in their capture history for each *Bartonella* group (A-E) were compared within each woodland site, to establish whether any broad host-*Bartonella* species associations were evident, and whether they were consistent across sites.

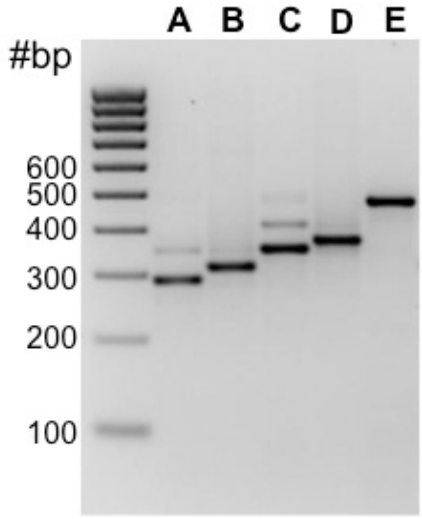

Figure 3.2: An Ethidium Bromide-stained agarose gel, showing the five categories (A-E) of *Bartonella* infection based on the size of the partial ITS region amplified by the primers of Telfer *et al*. (2005). Each amplicon size category broadly corresponds to the *Bartonella* species outlined in Table 3.1.

Table 3.2: Categorisation of *Bartonella* species infections based on the size of the partial ITS region amplified by the primers of Telfer *et al*. (2005). The *Bartonella* species that broadly correspond to each category are shown. These were determined by previous sequencing analysis of *Bartonella* isolates, whose identity had been determined by morphological and biochemical methods (R. Birtles, Pers. Comms).

pITS size category	Approximate pITS length (bp)	Putative *Bartonella* species
A	300	*B. doshiae* or *B. doshiae-like*
B	315	*B. grahamii*
C	350	*B. taylorii*
D	370	*B. birtlesii*
E	480	*B. rudakovii* or BGA

3.2.2.2 Genetic characterisation of the partial 16S-23S ITS region

The value of the above method for *Bartonella* identification lies in its relative low cost and high throughput nature. However, it is unable to differentiate between *Bartonella* species whose pITS amplicon lengths are very similar. For example, *B. rudakovii* and 'BGA' both have amplicons of approximately 480bp; similarly, the products of *B. doshiae* and an unnamed species previously referred to as *B. doshiae*-like (e.g. Telfer *et al*., 2007a) have pITS sequences that are both approximately 300bp long and so cannot be distinguished on amplicon size alone. Furthermore, this method cannot detect intra-*Bartonella* species genetic variability, which may be key to identifying specific associations between parasites and host species. To address these shortcomings, a subset of the diagnosed infections identified by the above method were subsequently genetically characterised at the pITS region.

A subset of blood samples positive for each *Bartonella* group (A-E) were selected for DNA sequencing. Samples were chosen randomly from each host species, and from across all trapping sessions, woodland sites and trapping grids. In this way, the identity and diversity of *Bartonella* infections within each group could be compared between host species and woodland sites. For the most part, only samples with a single PCR amplicon (i.e. not co-infected samples) were considered for sequencing, as limitations with capillary-based sequencing preclude concurrent characterisation of multiple PCR products within the same reaction. However, there were sometimes relatively few examples for which a *Bartonella* amplicon size group was present as a single infection within a particular host species at a particular site. In such instances, co-infected samples were used, by extricating the PCR product from the multiple products of a single sample after running on an agarose gel. The relevant DNA was then re-extracted prior to application in sequencing reactions using a MinElute® Gel Extraction Kit (Qiagen, UK), according to the manufacturer's protocol (published January 2011).

3.2.2.2.1 DNA sequencing protocol

For all directly sequenced PCR products, I first used ExoSAP to digest the reactions and remove excess unbound nucleotides and primers left over from the PCR. 5 μL of PCR product was added to 2 μL ExoSap reaction mixture containing 0.2 Units Shrimp Alkaline Phosphotase (New England Biolabs, UK), 1 Unit Exonuclease I (New England Biolabs, UK) and 10X SAP Reaction buffer. The mixture was then incubated for 45 minutes at 37 °C followed by 15 minutes at 80 °C. Purified PCR products were then sequenced in both directions using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies, Paisley, UK). 1 μL of each ExoSAP product (or pure gel-extracted DNA) was added to a 9 μL reaction mix containing 0.75 μL BigDye® v3.1 terminator sequencing dye (Applied Biosystems, UK), 5X BigDye® sequencing buffer and 0.32 pM of either the forward (*bigF*) or reverse (*bigR*) 2nd round PCR primers (see Chapter 2, Section 2.2 for primer sequences). Reaction mixtures were then exposed to 25 cycles of 96°C for 10 seconds, 50 °C for 5 seconds and 60 °C for 4 minutes. Following standard precipitation in 3 M sodium acetate, sequencing reaction products were re-suspended in Hi-Di™ formamide (Applied Biosystems, UK) and sequenced using an Applied Biosystems ABI PRISM® 3130xl Genetic Analyzer.

Sequence quality was assessed by visual inspection of chromatograms in Geneious Pro v5.6.6. Primer regions were trimmed and the forward and reverse sequences of each isolate were aligned using ClustalW within Geneious Pro, and verified visually, to give consensus pITS sequences for each *Bartonella* infection. All unambiguous consensus sequences were aligned using the default settings of ClustalW within Geneious Pro (gap open cost = 15; gap extension cost = 6.66), and unique sequence variants were identified by inspection of the resulting similarity matrix. Sequences that were 100% identical were grouped as a single unique *Bartonella* pITS variant. All unique variants were designated as belonging to a particular *Bartonella* species (*B. doshiae*, *B. doshiae*-like, *B. grahamii*, *B. taylorii*, *B. birtlesii*, *B. rudakovii* or *BGA*) based on their closest match to known *Bartonella* species within the NCBI nucleotide database using BLAST searches. Variants most closely matching the same *Bartonella* species were then aligned

(as described above) and mean pair-wise similarity and the number of base pair differences (including gaps and substitutions) between variants were calculated.

3.2.2.3 Comparison of *Bartonella* species identification methods

Rates of error associated with PCR-based species-level identification of *Bartonella* infections using only the size of the amplified pITS region were evaluated by comparison with those based on DNA sequences. Error rates were calculated, per pITS size category (A-E), as the proportion of infections designated as one species based on amplicon size that were more closely related to a different *Bartonella* species according to its DNA sequence.

3.2.3 Comparison of *Bartonella* variants across rodent hosts and woodland sites

A subset of *Bartonella* samples sequenced at the ITS region were used to assess whether the same or different assemblages of variants infected different host species, and whether different variants were present across the three woodland sites. A subset was used because rodent populations on two grids within each wood were exposed to experimental treatment from July to December in their respective trapping years (MW3, MW4, MF2, GOR, RH3 and RH4; see Chapter 5). This treatment aimed to perturb the transmission dynamics of *Bartonella* parasites within these rodent communities and therefore may have influenced which *Bartonella* variants were found to infect each host species. Comparisons of *Bartonella* variants across rodent hosts and woodland sites described below therefore considered infections characterised from rodents on unmanipulated treatment grids throughout the year (MW1, MW2, MF1 and RH2) and only those samples from treatment grids that were collected from May, June and July (i.e. pre-treatment).

3.2.3.1 Variant richness and diversity

For each *Bartonella* species, I calculated pITS variant richness (number of variants) and variant diversity (Shannon-Weiner diversity index) for wood mice and bank voles using the *'vegan'* package in R (v2.14.2). I then compared these measures across host species using data from all sites combined, and within each site separately, to broadly assess any between-host differences in variant assemblage in terms of richness and diversity. An unequal proportion of positive infections were characterised at the sequence level across *Bartonella* species, host species and sites; therefore I assessed whether this sampling bias may have affected the abundance of variants detected in each case. I used a method analogous to that presented in Streicker *et al*. (2010), whereby the number of variants per *Bartonella* species per host species per site was used as a response variable in a generalized linear model (GLM; log link for quasi-Poisson errors). I investigated the following explanatory variables: the proportion of positive samples that were characterised at the sequence level for each *Bartonella*-host-site combination (i.e. sequencing effort), and 2-way interactions between sequencing effort, host species and *Bartonella* species. These interaction terms were included to control for the fact that the rate at which novel variants are detected may vary across host and/or *Bartonella* species.

3.2.3.2 Broad host associations of variants within a *Bartonella* species group

I categorised each variant within a *Bartonella* species group as being found exclusively in a particular host species ("host-exclusive"), or being found in both wood mice and bank voles ("host-shared"). Note that I avoid the terms "host specific" and "host generalist" as they may imply a physiological or immunological compatibility (or lack thereof) between parasite and host, whereas I emphasise the associations I find may arise through exposure and/or compatibility limitations. For each *Bartonella* species, I then determined the proportion of infections characterised in each host species that were host-exclusive variants versus host-shared variants, as a broad measure of the degree of host-association within each *Bartonella* species group.

3.2.3.3 Phylogenetic relationships between variants of *Bartonella*

Using the DNA sequence alignments described in Section 3.2.2.2.1, phylogenetic relationships were inferred between ITS variants. Optimum nucleotide evolution models were first identified using the ModelTest function within MEGA 6.0 (Tamura *et al*., 2011), and unrooted maximum-likelihood trees were then estimated in MEGA 6.0. All positions with less than 95% site coverage were eliminated from the analysis. 1000 bootstrap re-samplings were performed in order to calculate percentage bootstrap support for tree topology. The analysis was carried out separately for each *Bartonella* species that was represented by multiple variants (*B. grahamii*, *B. taylorii* and *B. birtlesii*). This enabled relatedness between host-exclusive and host-shared variants to be assessed and, as such, possible directions of past host-switching events to be identified for each *Bartonella* species. Relatedness between variants found across different woodland sites was also assessed, to see if variants occurring in the same geographical location were more closely related to each other than to variants from elsewhere.

3.2.3.4 Comparison of *Bartonella* variant assemblages across host species

To determine whether the assemblage of *Bartonella* variants infecting conspecifics was more similar to each other than to those infecting individuals of a different host species, I performed a linear discriminant analysis (LDA) using the *"MASS"* package in R. If the parasite assemblages of wood mice and bank voles are significantly different, then an individual should be identifiable to host species based only on the variant of *Bartonella* with which they were infected (Venables & Ripley, 2002). Using the entire data set, a single linear discriminant (LD) was computed and the successful prediction of host species based on this LD was assessed by leave-one-out cross-validation, a procedure that iteratively uses each observation as a test of the model trained on the remainder of the dataset. More conservative assignment models were also constructed, trained on the host-parasite associations of only 75% of the data set. These models were then used to predict the host identity of the remaining 25% of the data (test set), and in each case the results were compared to a model of equal distribution of variants across individuals, constructed by random assignment of host species to observed variant

assemblages. The prediction successes of 1000 training sets based on true host-parasite associations and 1000 host-randomized training sets were then assessed using a χ^2 test. To ensure that the results were not biased by the inclusion of *Bartonella* species that were strictly host-exclusive (*B. doshiae*, *B. doshiae*-like, *B. rudakovii* and BGA), the same analysis was run with a reduced data set that included only the three *Bartonella* species that were host-shared, at least to some degree (*B. grahamii*, *B. taylorii* and *B. birtlesii*), and again for each of these species independently. Note that in all cases, variants observed on fewer than five occasions were omitted from the LDA, as their inclusion introduced computational problems when performing cross-validation.

3.2.3.5 Comparison of *Bartonella* variant assemblages across sampling sites

As data were collected from three woodland sites (MW, MFG and RH), any between-host differences in *Bartonella* variant assemblages may be confounded by differences in the parasite assemblages found at each site. Variant richness and diversity was therefore also compared across sites, and pair-wise similarities within and between site-exclusive variants were compared. An LDA was used to determine whether assemblages of variants found within rodent communities (wood mice and bank voles combined) were distinguishable between MW, MFG and RH, using woodland site as the grouping variable instead of host species. As three site groups were being compared, two LDs resulted in each case and the percentage of between group variance that each explained was determined. The ability to predict site identity based on the result of this LDA was then assessed as above using the test data sets.

3.2.3.6 Addressing non-independence of repeated samples from the same individuals

Multiple infections were sometimes sequenced from the same individual if sampled in multiple trapping sessions. If the same variant was detected on several occasions, this may arise from the same on-going infection rather than a newly acquired infection. Such non-independent data may artificially inflate the occurrence of particular pITS variants within a particular host species or site, and lead to spurious patterns of association. To address this possibility, all analyses were repeated using a reduced data set that included only a single record of a particular variant for each individual, even if it was detected in that individual multiple times.

3.3 Results

3.3.1 Blood samples collected

Blood samples were taken from 743 wood mice and 751 bank voles across the three woodland sites during the two-year study period. Most individuals were sampled only once (56% of wood mice (n=416) and 62% of bank voles (n=466)), but multiple blood samples were taken from 41% of individual rodents (n=612), resulting in a total of 1376 wood mouse and 1224 bank vole samples screened for the presence of *Bartonella* infections (Table 3.2).

Table 3.2: The number of individual wood mice and bank voles from which blood samples were taken at each woodland site throughout the study period. The numbers of blood samples taken from each rodent species at each site are also given. All blood samples were subsequently screened for *Bartonella* spp. infections. MW = Manor Wood, MFG = Maresfield & Gordale, RH = Rode Hall.

Site	Wood Mice		Bank Voles	
	# individuals	# samples	# individuals	# samples
MW	303	587	377	615
MFG	168	496	190	311
RH	272	293	184	298
Total	***743***	***1376***	***751***	***1224***

3.3.2 *Bartonella* infections as characterised by the length of the pITS region

The genus-specific partial ITS *Bartonella* PCR assay detected infections in 824 wood mouse blood samples (60%) and 595 bank vole blood samples (47%) across the three sites, with co-infections in 190 (23%) and 91 (15%) of the positive mouse and vole samples respectively. Infections with all *Bartonella* groups (A-E) were found at least once in individuals of both host species at all sites (Figure 3.3; Table 3.3). Absolute numbers of positive samples found in each host species at each site (which includes multiple samples from some individuals) are shown in the appendix (Table A3.1; Figure A3.1).

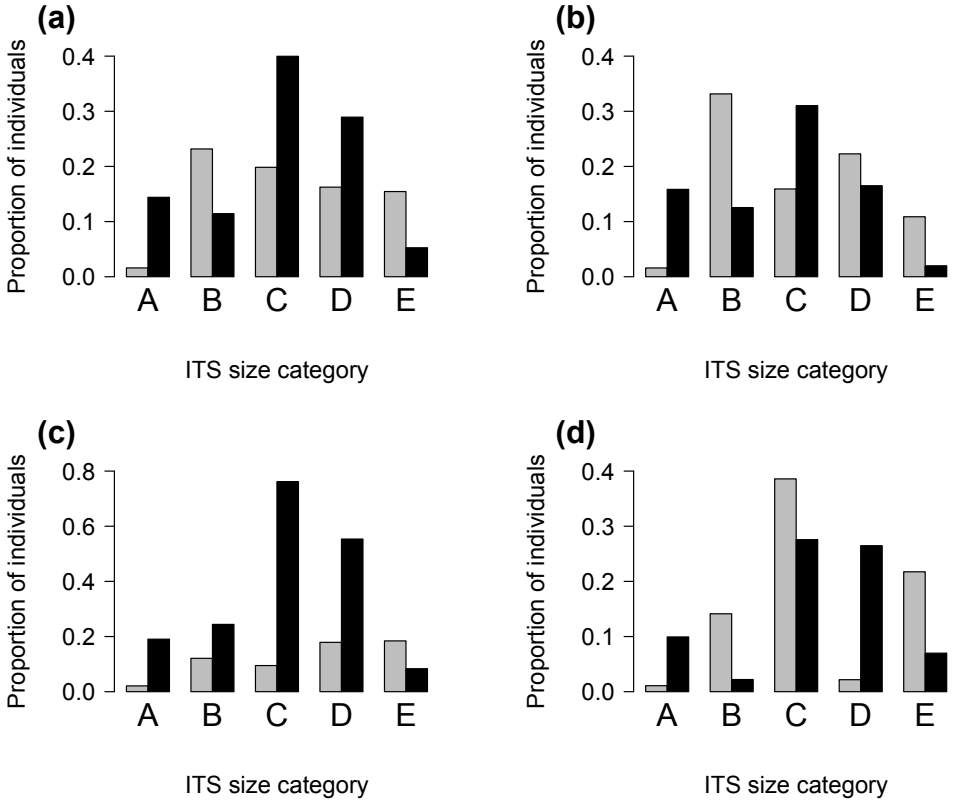

Figure 3.3: The proportion of individual wood mice and bank voles that tested positive for infections with each *Bartonella* pITS size group on at least one capture for (a) all sites combined, (b) Manor Wood, (c) Maresfield & Gordale, and (d) Rode Hall. pITS size groups relate to the following putative *Bartonella* species: A = *B. doshiae* or *B. doshiae*-like, B = *B. grahamii*, C = *B. taylorii*, D = *B. birtlesii* and E = *B. rudakovii* or BGA. Grey bars = Bank Voles, Black bars = Wood Mice

Table 3.3: The number of wood mice and bank voles that tested positive at least once for infection with each *Bartonella* pITS size group (A-E) at each woodland site. Chi-squared values indicate whether the proportion of each host species infected with each *Bartonella* group were significantly different overall and within each site separately. All Chi-squared values were significant ($p<0.01$), except where indicated (NS). pITS size groups relate to the following putative *Bartonella* species: A = *B. doshiae* or *B. doshiae*-like, B = *B. grahamii*, C = *B. taylorii*, D = *B. birtlesii* and E = *B. rudakovii* or BGA. Numbers in brackets are the number of wood mice and bank voles captured.

pITS group	All sites			MW			MFG			RH		
	WM (743)	BV (751)	χ^2	WM (303)	BV (377)	χ^2	WM (168)	BV (190)	χ^2	WM (272)	BV (184)	χ^2
A	107	12	83.5	48	6	46.7	32	4	28.3	27	2	14.4
B	85	174	35.9	38	125	39.2	41	23	9.2	6	26	23.9
C	297	149	72.3	94	60	21.9	128	18	164.3	75	71	6.1
D	215	122	34.4	50	84	3.54^{NS}	93	34	54.7	72	4	46.6
E	39	116	41.8	6	41	20.7	14	35	7.7	19	40	21.2

When considering data from all sites combined, group A (*B. doshiae* or *B. doshiae*-like), group C (*B. taylorii*) and group D (*B. birtlesii*) infections were found on at least one sampling occasion in a higher proportion of wood mice compared to bank voles. In contrast, group B (*B. grahamii*) and group D (*B. rudakovii* or BGA) infections were found in a higher proportion of bank voles (Table 3.3; Figure 3.3a). However, these patterns of relative prevalence across host species varied between woodland sites for group B, C and D, which makes it difficult to draw broad conclusions about host-parasite associations (Table 3.3; Figure 3.3b-d). Furthermore, although patterns were consistent across sites for group A and group E infections, these *Bartonella* groups represent multiple putative *Bartonella* species. It is therefore difficult to conclusively assess any host-*Bartonella* associations without further genetic characterisation of infections.

3.3.3 Genetic characterisation of *Bartonella* infections at the pITS region

In total, unambiguous pITS sequences were obtained for 441 wood mouse *Bartonella* infections and 390 bank vole infections. This included 53 isolates that were from *Bartonella* co-infections. As a result, sequences were ascertained for a high proportion of infections representing all *Bartonella* groups (A-E) from both host species at each site (>50% in most cases) (Table 3.4). Twenty-six unique sequences were identified, and all were at least 99% similar to sequences of known *Bartonella* species within GenBank (Table 3.5).

Table 3.4: The total number of blood samples that were positive for each *Bartonella* pITS group (A-E) from each host species at each site. The number and percentage of isolates subsequently sequenced at the pITS region in each case are given.

pITS size category (Putative *Bartonella* species identity)	Wood	Wood Mice		Bank Voles	
		#Infections	#sequenced	#Infections	#sequenced
A (***B. doshiae*** **or** ***B. doshiae*****-like)**	MW	66	16 (24%)	6	6 (100%)
	MFG	52	21 (40%)	4	0 (0%)
	RH	36	13 (36%)	2	2 (100%)
	Total	154	50 (32%)	12	8 (67%)
B (***B. grahamii***)	MW	49	23 (47%)	146	87 (60%)
	MFG	52	36 (69%)	26	22 (85%)
	RH	7	6 (86%)	28	22 (79%)
	Total	108	65 (60%)	200	131 (66%)
C (***B. taylorii***)	MW	148	70 (47%)	67	46 (69%)
	MFG	167	114 (68%)	19	17 (89%)
	RH	96	55 (57%)	99	78 (79%)
	Total	411	239 (58%)	185	141 (76%)
D (***B. birtlesii***)	MW	68	10 (15%)	101	24 (24%)
	MFG	124	24 (19%)	42	16 (38%)
	RH	98	19 (19%)	4	4 (100%)
	Total	290	53 (18%)	147	44 (30%)
E (***B. rudakovii*** **or BGA)**	MW	8	7 (88%)	48	37 (77%)
	MFG	17	11 (65%)	46	14 (33%)
	RH	28	15 (54%)	56	15 (25%)
	Total	53	33 (62%)	150	66 (44%)

Table 3.5: The twenty-six *Bartonella* partial 16S-23S ITS sequence types detected in this study. Sequence types are grouped into *Bartonella* species groups based on their closest match to known *Bartonella* species within GenBank. The accession numbers, source and description of these closest matches are given, along with percentage identity and coverage.

***Bartonella* species**	**pITS variant***	**# base pairs**	**pITS size category**	**Accession # of closest BLAST result**	**% Identity**	**% Coverage**
B. doshiae	Type 01	292	A	AJ269786.1	99	100
B. doshiae-like†	Type 12	293	A	AJ269792.1	100	100
B. grahamii	Type 02	314	B	KC907382.1	100	100
	Type 04	315	B	AJ269785.1	99	100
	Type 09	315	B	AJ269790.1	100	100
	Type 10	315	B	AJ269785.1	100	100
	Type 27	315	B	AJ269785.1	99	100
B. taylorii	Type 05	352	C	HM596435.1	100	100
	Type 06	352	C	AJ269788.1	99	100
	Type 07	352	C	DQ155391.1	99	100
	Type 11	352	C	AJ269788.1	100	100
	Type 13	352	C	DQ155391.1	99	100
	Type 14	352	C	KC907383.1	100	100
	Type 16	353	C	KC907384.1	100	100
	Type 20	351	C	AJ269784.1	100	100
	Type 21	351	C	AJ269796.1	100	100
	Type 29	352	C	KC907384.1	99	100
B. birtlesii†	Type 03	370	D	KC907381.1	100	100
	Type 15	370	D	AJ269787.1	99	100
	Type 17	370	D	AJ269791.1	100	100
	Type 22	351	C	KC907380.1	100	100
	Type 23	370	D	AJ269787.1	100	100
	Type 25	391	D	AJ269794.1	99	100
	Type 26	389	D	AJ269794.1	99	100
B. rudakovii	Type 08	461	E	EF682087.1	100	100
BGA	Type 24	466	E	DQ155376.1	100	100

† Pers. Comms. with Richard Birtles confirmed that "N40" was subsequently named *B. birtlesii* and that sample "wbs11" was subsequently referred to in their papers and others (e.g. Telfer *et al*., 2007a) as *B. doshiae*-like.

* pITS variant Type designations are arbitrary labels assigned to the variants found within this study.

3.3.3.1 Overview of variants identified within each *Bartonella* pITS group

As anticipated, pITS amplicons of ca. 300bp (pITS size group A) represented 2 distinct sequence variants that were identified as *B. doshiae* (Type 01; 259bp) and *B. doshiae*-like (Type 12; 260bp) (Table 3.5). An alignment of these two variants found them to be only 87.7% similar, with 32 base pair differences interspersed along the length of this region. Similarly, amplicons of ca. 480bp (pITS size group E) represented either *B. rudakovii* (Type 08) or BGA (Type 24), which were only 88.3% similar.

Several sequence variants were also identified within pITS size categories that represent a single putative *Bartonella* species (groups B, C and D). Five sequences were 314-315bp (group B) in length and all closely matched to *B. grahamii* (Type 02, Type 04, Type 09, Type 10, Type 29) (Table 3.5). Mean pair-wise similarity between *B. grahamii* sequences was 99.0%, with between only one and four single nucleotide base differences between each pair (Table 3.6a).

Eleven sequences were 351-353bp in length (group C); ten closely matched *B. taylorii* (Type 05, Type 06, Type 07, Type 11, Type 13, Type 14, Type 16, Type 20, Type 21, Type 29), but one was more similar to *B. birtlesii* (Type 22) indicating a possible source of error in identification based on pITS size alone (Table 3.5). Amongst the ten sequence variants most closely related to *B. taylorii*, mean pair-wise similarity was 98.3%, with a maximum of nine single base differences between pairs (Table 3.6b).

Along with Type 22, a further six variants matched to *B. birtlesii*; Four were 370bp in length and so fell within the group D size category (Type 03, Type 15, Type 17, Type 23) and two were slightly larger with lengths of 389bp (Type 26) and 391bp (Type 25) (Table 3.5). Mean pair-wise similarity was lower amongst variants of the *B. birtlesii* group compared to the *B. grahamii* and *B. taylorii* groups, being just 94.3%. The number of nucleotide differences between pairs of variants ranged between 1 and 43 (Table 3.6c). Type 22 has a much shorter sequence, and Types 25 and 26 have longer sequenced compared to all other *B. birtlesii* types, indicating past insertion or deletion of relatively large portions of DNA in relation to this type.

Table 3.6: Pair-wise similarities between different pITS variants that most closely match to (a) *B. grahamii*, (b) *B. taylorii* and (c) *B. birtlesii*. Numbers in black are percentage pair-wise similarities between sequences. The absolute numbers of nucleotide differences (including substitutions and indels) between sequence pairs are shown in grey italics. Sequence types are represented by the prefix "T" followed by their reference number as given in Table 3.5.

(a)

	T 02	**T 04**	**T 27**	**T 09**
T 04	98.6 *4*			
T 27	98.6 *4*	98.6 *4*		
T 09	98.9 *3*	98.9 *3*	98.9 *3*	
T 10	99.3 *2*	99.3 *2*	99.3 *2*	99.6 *1*

(b)

	T 06	**T 07**	**T 05**	**T 11**	**T 14**	**T 13**	**T 16**	**T 2**	**T 21**
T 07	99.1 *3*								
T 05	99.1 *3*	99.4 *2*							
T 11	99.7 *1*	98.7 *4*	98.7 *4*						
T 14	98.4 *5*	98.7 *4*	98.7 *4*	98.1 *6*					
T 13	98.1 *6*	99.1 *3*	98.4 *5*	98.4 *5*	97.8 *7*				
T 16	98.1 *6*	98.4 *5*	98.4 *5*	98.4 *5*	97.8 *7*	98.1 *6*			
T 20	97.8 *7*	98.1 *6*	98.1 *6*	97.5 *8*	98.1 *6*	97.2 *9*	97.2 *9*		
T 21	98.1 *6*	98.4 *5*	98.4 *5*	97.8 *7*	98.4 *5*	97.5 *8*	97.5 *8*	99.7 *1*	
T 29	98.4 *5*	98.7 *4*	98.7 *4*	98.7 *4*	98.1 *6*	98.4 *5*	99.7 *1*	97.5 *8*	97.8 *7*

(c)

	T 03	**T 15**	**T 17**	**T 22**	**T23**	**T25**
T 15	96.2 *13*					
T 17	97.0 *10*	99.1 *3*				
T 22	91.4 *29*	94.1 *20*	93.8 *21*			
T 23	96.4 *12*	99.7 *1*	99.4 *2*	94.4 *19*		
T25	91.0 *32*	94.1 *21*	93.8 *22*	89.0 *39*	94.4 *20*	
T26	90.0 *36*	93.0 *25*	92.7 *26*	88.0 *43*	93.3 *24*	98.6 *5*

3.3.3.2 Potential error of *Bartonella* species identification based on length of pITS region

The sequencing of *Bartonella* infections at the pITS region indicated that identification of infections based on pITS length alone had some associated error (ranging from 0 to 14% of samples for each group; Table 3.7). Most of this error was apparently due to the mis-estimation of PCR amplicon size; when sequenced, the true length of amplified DNA sequences sometimes differed from the original estimate, and these true amplicon lengths concurred with species identity as revealed by DNA sequences. However, as mentioned above, several (16) Type 22 *B. birtlesii* infections were originally mis-identified as *B. taylorii* due to the shorter length of this pITS region, therefore operator error alone was not wholly responsible for incorrect *Bartonella* species identifications.

Two important findings emerged from a comparison of identification methods here. Firstly, contrary to original diagnoses, there were no group A infections (*B. doshiae* or *B. doshiae*-like) in bank voles at Maresfield & Gordale (these were in fact *B. grahamii*, based on sequence identity). Secondly, there were no group D infections (*B. birtlesii*) in bank voles at Rode Hall (these were in fact *B. taylorii*).

Table 3.7: The number of *Bartonella* infections within each pITS size category (A-E) that were sequenced at this genome region, and the number of times identification based on DNA sequence matched these original putative species categorisations. Where DNA sequencing revealed an infection to be of a different species to that originally perceived, identity according to DNA sequence is given. The number of times such mismatches occurred is given in brackets, and the overall number and percentage of incorrect original identifications within each pITS size category (error rate) has been calculated.

pITS size category	Putative *Bartonella* sp.	# Sequenced	Sequence ID	# Mismatches
A	*B. doshiae*/ *B. doshiae*-like	58	*B. grahamii* (5) *B. birtlesii* (2) *B. taylorii* (1)	8 (14%)
B	*B. grahamii*	196	BGA (1) *B. birtlesii* (3) *B. doshiae* (10) *B. taylorii* (2)	16 (8.2%)
C	*B. taylorii*	380	BGA (1) *B. birtlesii* (30) *B. grahamii* (8)	39 (10%)
D	*B. birtlesii*	97	*B. rudakovii* (2) *B. taylorii* (7)	9 (8.6%)
E	*B. rudakovii*/BGA	99	NA	0 (0%)

3.3.4 Comparison of *Bartonella* variants infecting wood mice and bank voles

A total of 550 *Bartonella* infections, characterised at the pITS region, were collected either from observation grids or from treatment grids prior to the onset of treatment and therefore used in the following analyses. This included 261 wood mouse infections and 289 bank vole infections. Note that two rare variants (Type 25 and Type 26; both classed as *B. birtlesii* and detected on a single occasion) were only found on treatment grids after the onset of treatment and are therefore not included in the following analyses.

3.3.4.1 Richness and diversity of *Bartonella* variants across host species

There was great variation in numbers of isolates per *Bartonella* pITS variant detected within each host species (Table 3.8.) With all *Bartonella* species combined, a greater richness and diversity of variants was found in wood mice compared to bank voles overall and at each site separately (Table 3.9). When *Bartonella* species were considered separately, greater variant richness and diversity was still found in wood mice for *B. birtlesii* and *B. taylorii* at all sites. However, the reverse pattern was evident for *B. grahamii*, with richness and diversity being greater in bank voles compared to wood mice. These patterns of variant richness and diversity were the same for the smaller data set data set that included only a single record of a particular variant for each individual, even if it was detected in that individual multiple times (Table A3.2).

Table 3.8: Number of isolates of each pITS variant sequenced from wood mice and bank voles at each woodland site (not including samples collected from populations exposed to experimental treatment; see Section 3.2.3). Variants in green were only found in bank voles; Variants in red were only found in wood mice; Variants in purple were found in both rodent species.

***Bartonella* sp.**	**pITS type**	**All sites**		**MW**		**MFG**		**RH**	
		WM	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**
B. doshiae-like	Type 12	40		20		13		7	
B. doshiae	Type 01		2		1				1
B. grahamii	Type 02		77		64		13		
	Type 04		14						14
	Type 27		1		1				
	Type 09	2	3			2	3		
	Type 10	20	6	7	2	13	4		
B. taylorii	Type 06		1						1
	Type 07		3						3
	Type 05	8	94	4	40	3	8	1	46
	Type 14	10	3			9		1	3
	Type 11	39		15		15		9	
	Type 13	4				4			
	Type 16	1				1			
	Type 20	61		12		27		22	
	Type 21	3		3					
	Type 29	5		1		4			
B. birtlesii	Type 03	2	41		23	1	18	1	
	Type 15	8				8			
	Type 17	6				6			
	Type 22	5		5					
	Type 23	24		4		7		13	
B. rudakovii	Type 08		44		26		9		9
BGA	Type 24	23		3		7		13	

Table 3.9: Variant richness (the number of different variants, *N*) and variant diversity (Shannon-Weiner diversity index, *H*) of each *Bartonella* species detected in wood mice (WM) and bank voles (BV) at all sites combined and at each site individually.

***Bartonella* sp.**	**All sites**				**Manor Wood**				**Maresfield & Gordale**				**Rode Hall**			
	N		***H***		***N***		***H***		***N***		***H***		***N***		***H***	
	WM	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**
All	17	12	2.18	1.62	10	7	1.89	1.37	15	6	2.23	1.63	8	7	1.70	1.00
B. doshiae	0	1	NA	0	0	1	NA	0	0	0	NA	NA	0	1	NA	0
B. doshiae-like	1	0	0	NA	1	0	0	NA	1	0	0	NA	1	0	0	NA
B. grahamii	2	5	0.30	0.77	1	3	0	0.18	2	3	0.39	0.89	0	1	NA	0
B. taylorii	8	4	1.10	0.14	5	1	0.98	0	7	1	1.16	0	4	4	0.84	0.23
B. birtlesii	5	1	1.29	0	2	1	0.69	0	4	1	1.23	0	2	0	0.26	NA
B. rudakovii	0	1	NA	0	0	1	NA	0	0	1	NA	0	0	1	NA	0
BGA	1	0	0	NA	1	0	0	NA	1	0	0	NA	1	0	0	NA

There was no association between the proportion of positive samples sequenced and the variant richness detected per *Bartonella* species-host-site combination (GLM: Proportion sequenced $F_{1,26} = 0.02$, $p = 0.90$; Figure 3.4), and this result was consistent across host species (GLM: Proportion sequenced*Host species $F_{1,26} = 0.08$, $p = 0.78$) and *Bartonella* species (GLM: Proportion sequenced**Bartonella* species $F_{5,26} = 0.40$, $p = 0.84$). Further sequencing of the positive samples collected is therefore unlikely to increase the number of *Bartonella* spp. variants found in each host species at each site, and so the assemblages detected would appear to be representative of the true patterns in the community.

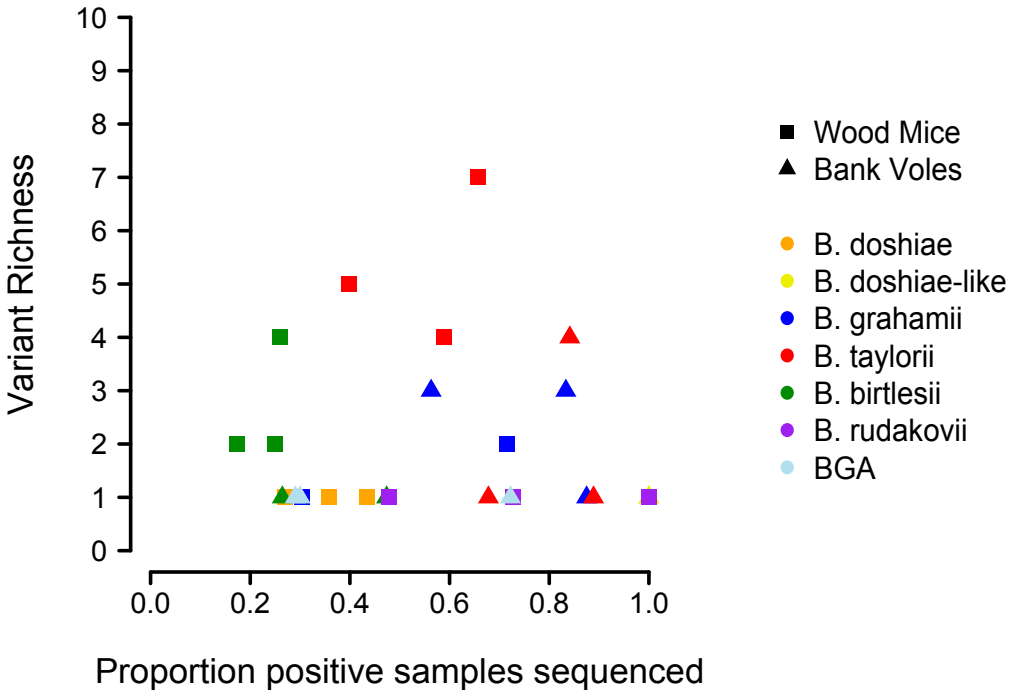

Figure 3.4: Relationship between the proportion of positive samples per *Bartonella* species that were sequenced per host species per site, and the number of variants for that *Bartonella* species that were detected. Symbols are colour-coded by *Bartonella* species, with different symbol shapes for host species. A GLM revealed no significant association overall or within each host species or *Bartonella* species subset.

3.3.4.2 Broad host associations of variants within a *Bartonella* species group

Bartonella species represented by a single pITS variant

Four *Bartonella* species were represented by single variants, and each was detected in a single host species. The single *B. doshiae* variant (Type 01) and the single *B. rudakovii* variant (Type 12) were bank vole-exclusive, while the single *B. doshiae*-like variant (Type 12) and the single BGA variant (Type 24) were wood mouse-exclusive.

Bartonella species represented by multiple pITS variants

For the three *Bartonella* species that were represented by multiple pITS variants (*B. grahamii, B. taylorii* and *B. birtlesii*), variants of the same species often differed in their associations with rodent hosts, with some found exclusively in a single host species and others found in both (Table 3.8). Each of these *Bartonella* species is discussed separately below.

Bartonella grahamii

B. grahamii variants were not wood mouse-exclusive, although the majority of wood mouse infections (~91%) constituted variants that were relatively rare in bank voles (Type 10). More than half of the *B. grahamii* variants (3/5) were bank vole-exclusive (Types 02, 04, 27), and these bank vole-exclusive variants constituted 91% of all characterised bank vole *B. grahamii* infections (Figure 3.5a).

Phylogenetic relationships between *B. grahamii* variants were poorly resolved, as bootstrap support for branches was always below 70% (in fact below 35% in all cases) (Figure 3.6a). It was therefore not possible to deduce relationships between variants infecting different host species, and so the direction of past host-switching events could not be inferred.

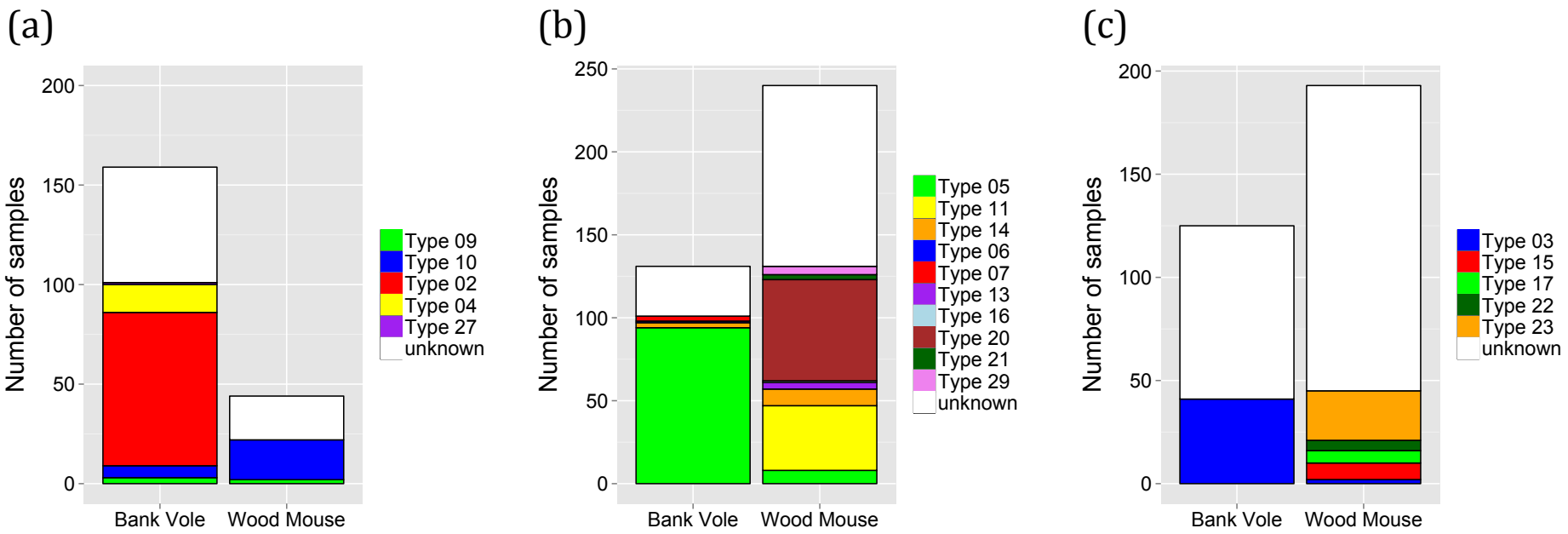

Figure 3.5: The number of each (a) *B. grahamii* (b) *B. taylorii* and (c) *B. birtlesii* variant detected within wood mice and bank voles across all woodland sites. Colour coding represents different variants within each *Bartonella* species group; separate keys are provided for variants within each *Bartonella* species. Infections that were not sequenced are classed as "unknown" variants. Note that classification of "unknown" variants into their respective *Bartonella* species groups is based on pITS length alone, and therefore may be associated with slight error as outlined in Table 7. Note that the y-axis range varies across *Bartonella* species.

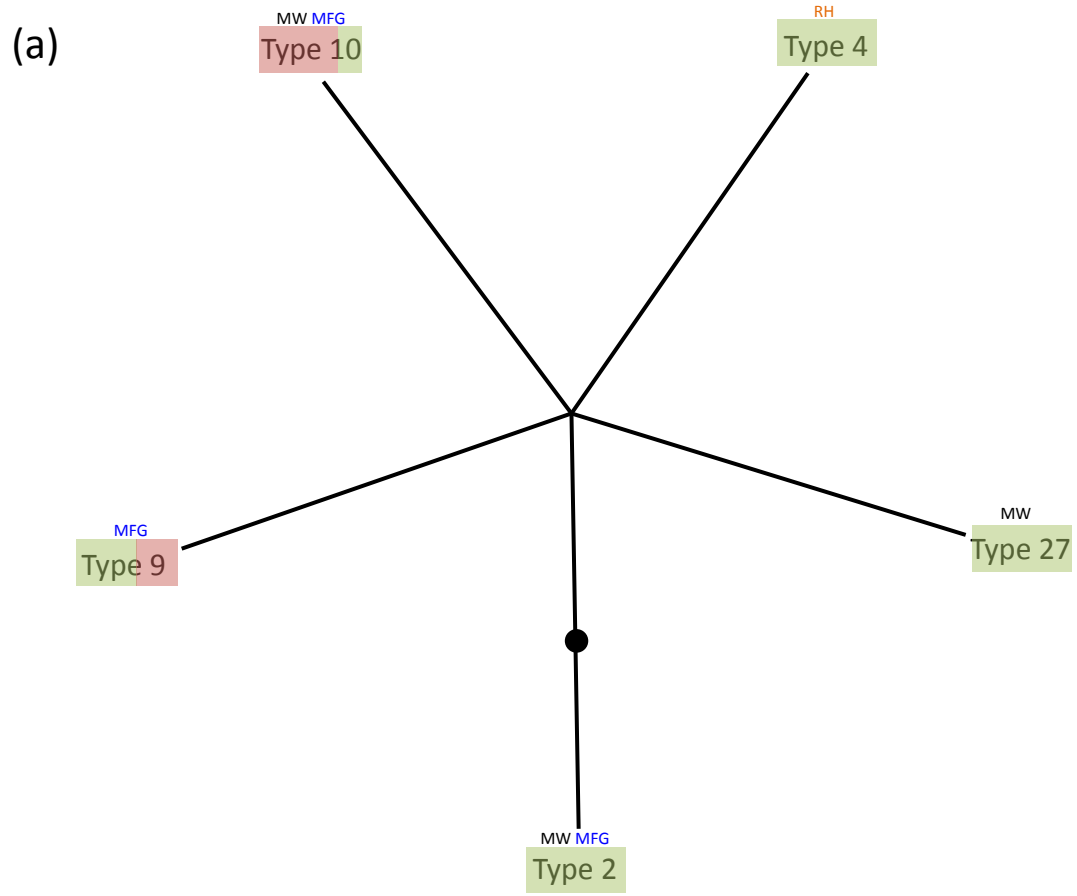

Figure 3.6: Unrooted maximum-likelihood phylogenetic trees showing the relationships between pITS variants of (a) *B. grahamii*, (b) *B. taylorii* and (c) *B. birtlesii*. The Tamura 3-parameter model of nucleotide evolution was used in all cases. Percentage bootstrap support for clades based on 1000 re-samplings is shown in italics. Only clades with> 70% bootstrap branch support are presented. Branch lengths are proportional to the number of substitutions per site. Indels are indicated on branches where they occur (● = deletion, ● = insertion). Variant names are colour-coded according to the proportion of isolates found in wood mice (red) and bank voles (green). The woodland locations where each variant was found are also shown: MW = Manor Wood, MFG = Maresfield & Gordale, RH = Rode Hall. Figure continued on next page.

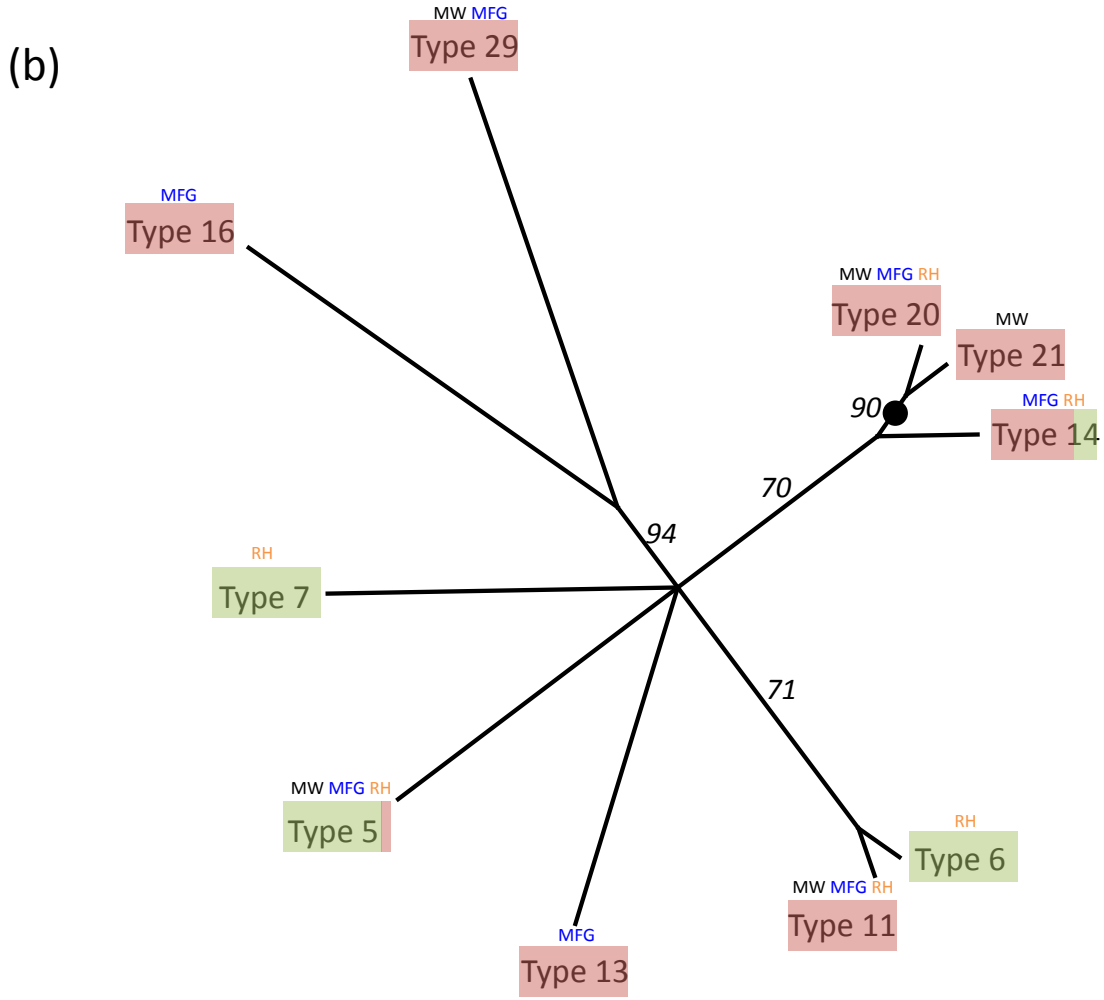

Figure 3.6: Continued from previous page.

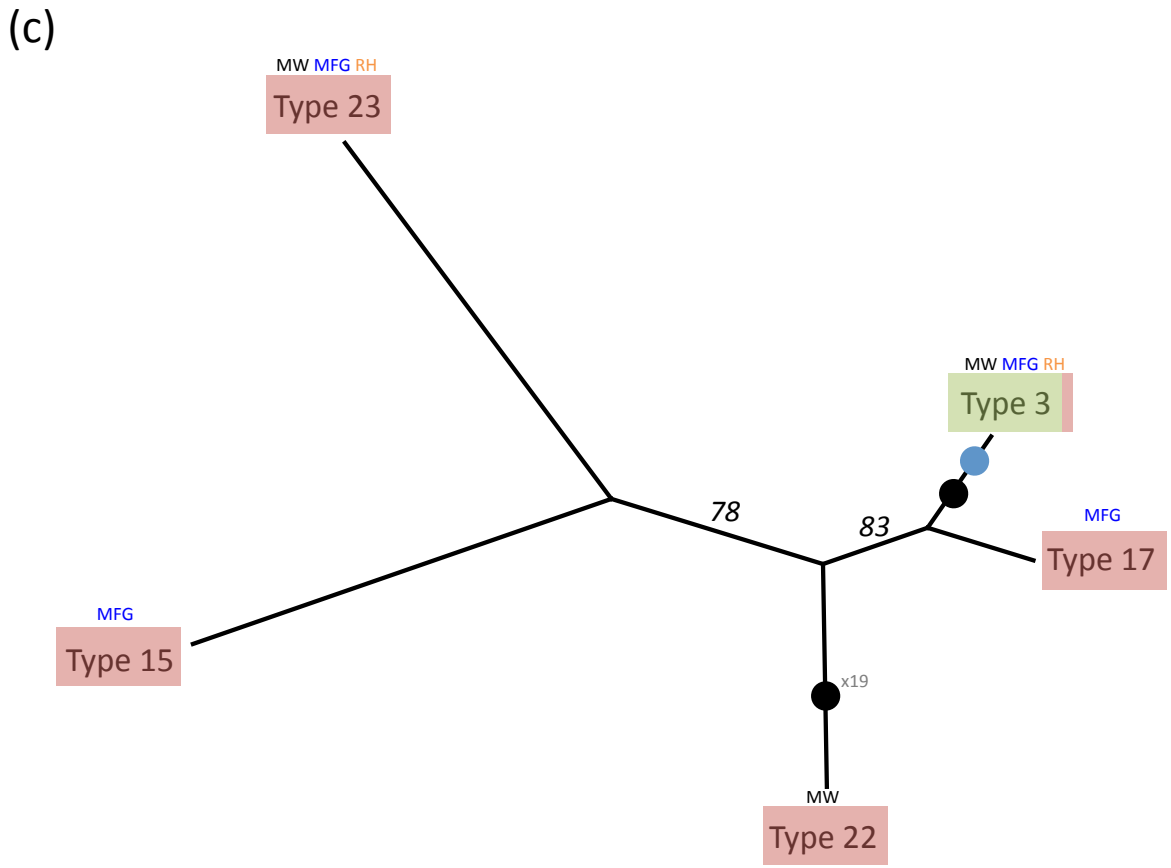

Figure 3.6: Continued from previous page.

Bartonella taylorii

More than half (6/10) of the *B. taylorii* variants were wood mouse-exclusive (Types 11, 13, 16, 20, 21, 29), only 2/10 were bank vole-exclusive (Types 06 and 07) and 2/10 were host-shared (Types 05 and 14). The majority of *B. taylorii* infections characterised from wood mice (86%) were of wood mouse-exclusive variants. In contrast, the majority of characterised bank vole *B. taylorii* infections (96%) were host-shared variants, with only 4% being bank vole-exclusive variants (Figure 3.5b).

An analysis of phylogenetic relationships between variants of *B. taylorii* found strong support for a clade consisting of Type 20 and Type 21, which are both wood mouse-exclusive variants; however, these variants formed part of a larger clade, which also included Type 14, a host-shared variant, possibly suggesting a previous host-switching event. However, two other wood mouse-exclusive variants formed a separate well-supported clade (Type 16 and Type 29), and there was strong support for another separate clade that included both a wood mouse-exclusive (Type 11) and bank vole-exclusive (Type 6) variant, which gives no indication of the direction of any host-switching between these rodent species. In addition, the phylogenetic relationships of several other variants could not be resolved (Type 7, Type 5 and Type 13), including the variant that was found to infect bank voles most often (Type 5). It is therefore difficult to establish when, how often, and the direction of host-switching events that may have occurred for *B. taylorii* between wood mice and bank voles.

Bartonella birtlesii

Finally, the majority of *B. birtlesii* variants were wood mouse-exclusive (4/5) and none were bank vole-exclusive. However, while the single *B. birtlesii* variant detected in bank voles (Type 03) was also found in wood mice (i.e. host-shared), this variant constituted only 4% of the wood mouse *B. birtlesii* infections characterised, with the majority being of variants that were wood mouse-exclusive (Figure 3.5c).

An analysis of the phylogenetic relationships between *B. birtlesii* variants found strong support for a clade consisting of Type 17, a wood mouse-exclusive variant, and Type 3, the only variant found in bank voles. It is difficult to infer the direction of any host-

switching event here, as there are no strict bank vole-exclusive *B. birtlesii* variants. However, all other variants are wood mouse exclusive, and two of these form a separate, well-supported cluster (Type 23 and Type 15). This indicates that even if a host-switching event did occur at some point from bank voles to wood mice, it was not necessarily a pre-requisite for wood mouse infection with *B. birtlesii*. The relationship of the final variant, Type 22, with other *B. birtlesii* variants could not be resolved.

3.3.4.3 Linear Discriminant Analysis of *Bartonella* variant assemblages infecting wood mice and bank voles

The LDA demonstrated that wood mice and bank voles (all sites combined) were infected with distinguishable assemblages of *Bartonella* variants (Figure 3.7). Leave-one-out cross validation of the full model correctly classified 96.3% of individuals to species. In a more conservative test, the assignment models trained from a 75% subset of the true data (n = 581) correctly assigned significantly more individuals than the randomized training sets (true data: 98.0% [95% CI: 97.9-98.1]; random data: 72.5% [95% CI: 71.4-73.6]; χ^2 = 259, *d.f.* = 1, $p < 0.001$). Wood mouse and bank vole *Bartonella* assemblages were consistently distinguishable even when considering only variants of the three *Bartonella* species that were host-shared to at least some degree (*B. grahamii*, *B. taylorii* and *B. birtlesii*), and when each of these species were considered independently (Table A3.3). Furthermore, all LDA results were consistent when using the smaller dataset that only ever included a single record of a variant per individual, even if found multiple times in the same individual (Table A3.4).

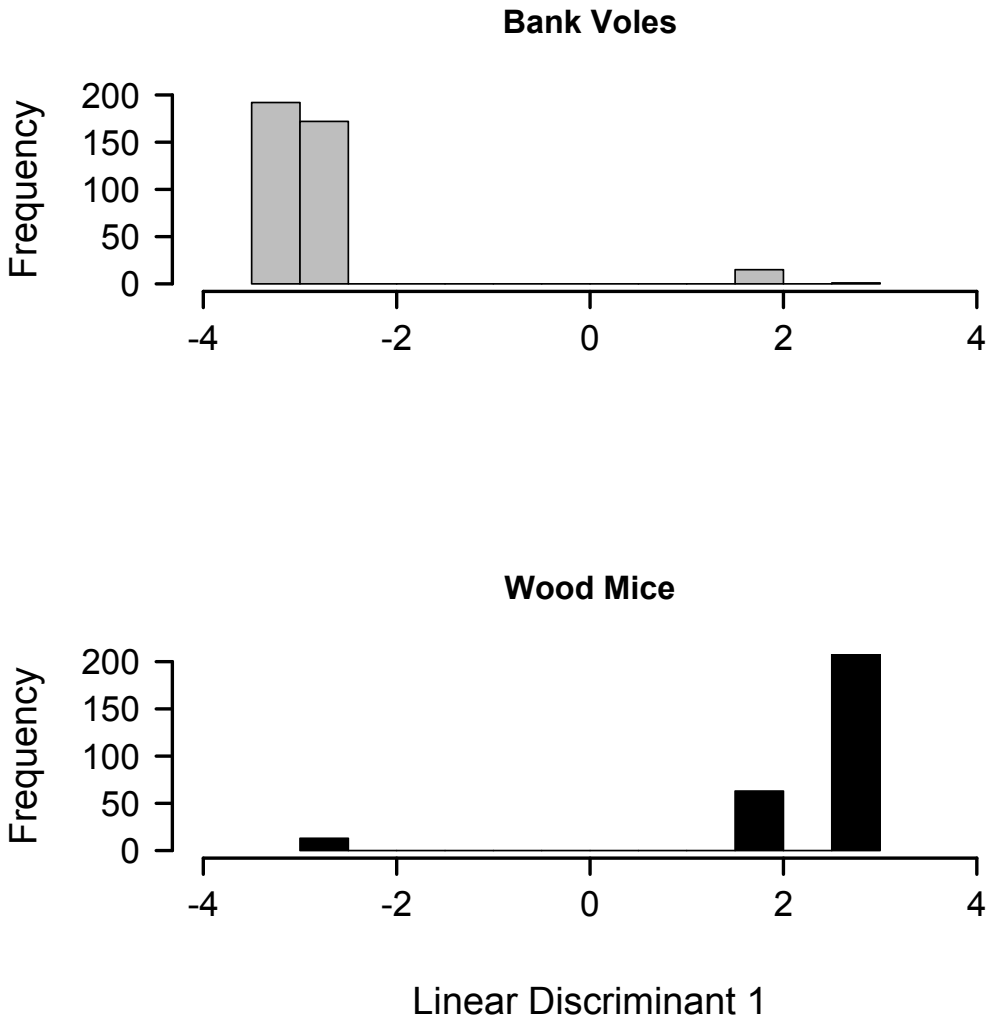

Figure 3.7: Linear Discriminant Analysis of *Bartonella* pITS variant assemblage using host species as a grouping factor. The distribution of linear discriminant values calculated for each (a) bank vole and (b) wood mouse sample for which *Bartonella* infections were characterised to pITS variant level are shown. There is clear discrimination between the majority of values calculated for each host species, indicating that *Bartonella* pITS variant assemblages associated with each host species do not significantly overlap.

3.3.5 Comparison of *Bartonella* variant assemblages across sites

3.3.5.1 Broad site associations of variants within a *Bartonella* species group

There were differences in the assemblages of *Bartonella* variants found within the three different woodland sites. Overall, and for *B. taylorii* and *B. birtlesii* individually, variant richness and diversity were lowest at RH in both host species, and for *B. grahamii* in wood mice (Table 3.9). No *B. birtlesii* infections were found in any bank voles at this site, even though the variant infecting bank voles at the other sites (Type 03) was present in a wood mouse at RH. Again, these patterns of variant richness and diversity were consistent when variants found on multiple occasions from the same individual were included only once in the data set (Table A3.2). Despite these differences, the assemblages of *Bartonella* variants found at the three sites were broadly similar.

Bartonella species represented by a single pITS variant

The single variants of *B. doshiae*-like , *B. rudakovii* and BGA were found at all sites.

Bartonella species represented by multiple pITS variants

B. grahamii

The majority of *B. grahamii* infections at MW (99%) and MFG (86%) were of two site-shared variants (Types 10 and 02), but all of the *B. grahamii* infections at RH were of Type 04, which was exclusive to this site (Figure 3.8a). As the phylogenetic relationships between *B. grahamii* variants were poorly resolved (Figure 3.6a), it was not possible to infer relationships between variants found at different woodland sites.

B. taylorii

Three *B. taylorii* variants (Types 05, 11, and 20) were found at all sites and consistently represented the majority of *B. taylorii* infections (95% at MW, 75% at MFG, 79% at RH). Site-exclusive variants accounted for a small proportion of infections at each site (Figure 3.8b). The two bank vole-exclusive variants (Types 06 and 07) were only found at RH. The phylogenetic analysis indicated no obvious clustering of *B. taylorii* variants according to woodland site (Figure 3.6b).

B. birtlesii

Two *B. birtlesii* variants (Types 03 and 23) were found at all sites and consistently represented the majority of infections (84% at MW, 65% at MFG, 100% at RH; Figure 3.8c). Again, the phylogenetic analysis indicated no obvious clustering of *B. birtlesii* variants according to woodland site (Figure 3.6c).

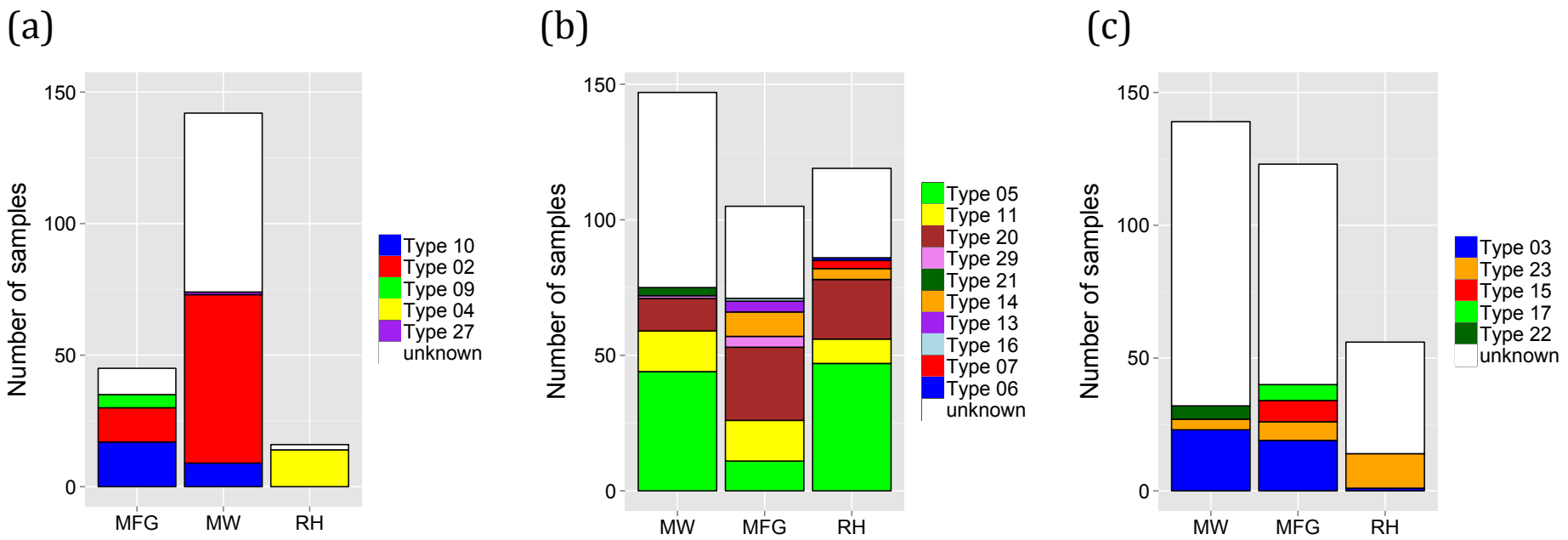

Figure 3.8: The number of each (a) *B. grahamii* (b) *B. taylorii* and (c) *B. birtlesii* variant detected within all rodents (wood mice and bank voles combined) at each woodland site. Colour coding represents different variants within each *Bartonella* species group; separate keys are provided for variants within each *Bartonella* species. Infections that were not sequenced are classed as "unknown" variants. Note that classification of "unknown" variants into their respective *Bartonella* species groups is based on pITS length alone, and therefore may be associated with slight error as outlined in Table 7. MW = Manor Wood, MFG = Maresfield & Gordale Woods, RH = Rode Hall.

3.3.5.2 Linear Discriminant Analysis of *Bartonella* variant assemblages infecting rodent communities at different sites

The differences between sites were confirmed by the spatial LDA (Figure 3.9). Based on two linear discriminants, leave-one-out cross validation of the full model correctly classified only 57.8% of individuals to site, although the more conservative assignment model trained from a 75% subset of the true data correctly assigned significantly more individuals in the test set than the randomized training sets (true data: 80.2% [95% CI: 79.6-80.8]; random data: 44.7.0% [95% CI: 43.3-46.1]; $\chi^2 = 269$, $d.f. = 1$, $p < 0.001$). However, the prediction success of the site-based linear discriminants was consistently lower than that for host species, using all subsets of the data (compare Tables A3 & A5).

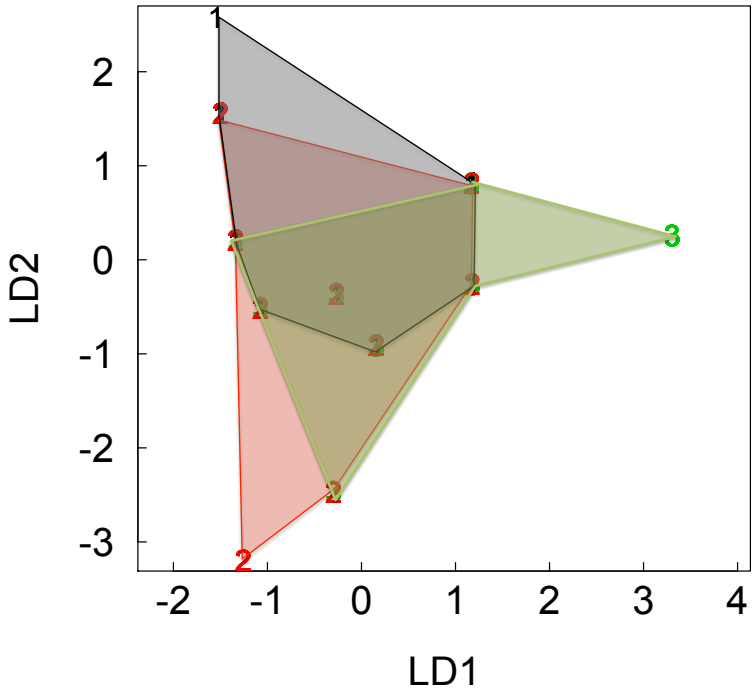

Figure 3.9: Linear Discriminant Analysis of *Bartonella* pITS variant assemblage using woodland site as a grouping factor. The plot places each sample in 2-dimensional space according to the two linear discriminant values calculated (LD1 and LD2). Sites are coded as follows: MW = 1 (black), MFG = 2 (red), RH = 3 (green). Polygons were drawn by eye, and indicate the 2D linear discriminant space relating to each site (same colour coding). There is no clear discrimination of *Bartonella* variant assemblages between sites.

3.4 Discussion

Sequencing of rodent *Bartonella* infections at a fragment of the ITS region revealed substantial variation, often within the same *Bartonella* species. Importantly, variants displayed a range of host associations, with many being found exclusively in either wood mice or bank voles, and relatively few being found in both host species. As a result, there was clear differentiation between the parasite assemblages infecting each host species. Importantly, several *Bartonella* species that were previously thought to be multi-host generalists were found to comprise a complex of relatively host-specific variants, indicating that between species transmission is less frequent than previously thought. This finding accompanies previous work that identified limited between-species transmission of cowpox virus infections in wild rodent communities (Begon *et al*., 1999), and suggests that covert host-specialism of apparently multi-host parasites may be a general phenomenon.

A lack of *Bartonella* spp. transmission between wood mice and bank voles may result from either ecological or physiological incompatibilities (or both) of hosts and parasites (Combes, 2001). Rodent *Bartonella* parasites are transmitted between individuals via the feeding behaviours of fleas (Bown *et al*., 2004; Morrick *et al*., 2011). It is therefore possible that specific associations between rodent hosts and flea species preclude the transfer of fleas between wood mice and bank voles, creating a flea-mediated ecological barrier to between-species transmission of *Bartonella*, and resulting in genetic drift of parasite populations through isolation. Previous studies have, however, found that several of the same flea species infest sympatric wood mice and bank voles (e.g. Telfer *et al*., 2005), suggesting that specific associations between hosts and fleas are not likely. A more in-depth investigation of the assemblage of flea vectors within this study system will be crucial for understanding their role in *Bartonella* transmission and host specificity (see Chapter 4 for more details).

Even if flea vectors are host generalists, there may be small-scale structuring of the flea community, such that discrete subsets of the flea community circulate independently within each host species population, therefore limiting the between-species transmission of *Bartonella*. Jousson *et al*. (2000) found that differences in host ecology are a possible

driver of covert host-specificity of passively transmitted Digenean parasites of sympatric teleost fishes, as the distribution of different parasite variants were associated with the differing diets of host species. In this rodent system, the distribution of fleas is likely to rely on the behaviours of the hosts, as they are mostly nest-dwelling species that feed opportunistically on hosts entering these nests (Marshall, 1981; Krasnov, 2008). In contrast to other ectoparasitic vectors whose movement patterns are relatively uncoupled from their vertebrate hosts (e.g. *Plasmodium*-transmitting mosquitoes), flea-mediated transmission of *Bartonella* is therefore likely to depend on the density and movement of hosts rather than the vector, in a situation analogous to parasite transmission via ticks (Randolph, 1998). Transfer of fleas between host species is likely to require regular contact between wood mice and a flea-infested bank vole nest, or vice versa. However, differences in activity patterns (nocturnal wood mice versus crespuscular bank voles; Greenwood, 1978), extent of spatial movement (larger home range for wood mice compared to bank voles; Crawley, 1969) and in the diet and habitat vegetation preferences (largely granivorous wood mice compared to folivorous/granivorous bank voles; Watts, 1968; Canova, 1993) of these rodents may result in such contacts being rare. Where variants were found in both host species, they were usually more common in one host that the other (e.g. *B. grahamii* variant Type 09 was more common in wood mice than bank voles, and *B. taylorii* variant Type 05 and *B. birtlesii* variant Type 03 was more common in bank voles than wood mice), which may indicate spillover transmission (Power & Mitchell, 2004) of these variants given rare opportunities for flea transfer.

Rather than an ecological barrier to between-species transmission, differences between *Bartonella* parasites infecting wood mice and bank voles may arise from physiological barriers to transmission between host species. For example, Vassier-Taussat *et al*. (2010) demonstrated that host specificity of different *Bartonella* species across distantly related mammalian hosts is associated with the ability of bacteria to adhere to the erythrocytes of their hosts, a process mediated by a cluster of genes within the Trw-type IV secretion system. It possible that the host-exclusive pITS variants identified here are also distinguishable at loci related to this host-specificity operon, or at loci related to evasion of host-specific immune defences. Of course such physiological host-parasite incompatibilities may have evolved as a consequence of ecological isolation from particular host species in the past, as parasites are likely to become adapted to exploit

host species with which they have most contact (Poulin *et al*., 2008). An investigation of the genetic diversity of such potential host-specificity loci, coupled with experimental reciprocal infections of naturally occurring variants, would help to elucidate the cause and effect of the genetic variation identified here.

Several previous studies have found significant genetic diversity within *Bartonella* parasites, which may be the result of adaptation to different host species, or as a co-evolutionary response to enable evasion of host immunity. Studies in Europe and North America have found that different *Bartonella* variants continually replace one another within the same host individual, thus supporting this idea (Buffet *et al*., 2013; Kosoy *et al*., 2004, Telfer *et al*., 2007a, Paziewska *et al*., 2012). Such diversity is thought to have arisen via homologous recombination between closely related strains (Paziewska *et al*., 2012; Berglund *et al*., 2010a), which may be promoted by vector transmission. Co-infection of multiple *Bartonella* variants within flea vectors has been demonstrated (e.g. Abbot *et al*., 2007), and lateral genetic transfer between them may give rise to novel variants and an increase in *Bartonella* diversity. Such recombination events may explain why multiple pITS variants of the same *Bartonella* species were found to infect the same host species in this study, if recombination acts to alter the DNA sequence at this non-coding region without altering the function of any genes related to host specificity.

Interestingly, variant richness of *Bartonella* differed across host species and geographical location. Overall, wood mice were found to harbour a greater number of *Bartonella* variants compared to bank voles. This may be a reflection of the greater average home range size of wood mice compared to bank voles (Crawley, 1969; Carslake *et al*., 2005), which may act to increase the diversity of *Bartonella* variants. Firstly, it may increase the rate of encounter and colonisation of new parasite variants from more distant populations of wood mice, in accordance with the theory of island biogeography (MacArthur & Wilson, 1967; Poulin, 1997). Furthermore, contact with a greater number of individuals may promote higher rates of co-infection within wood mouse flea vectors, thus increasing opportunities for within-vector recombination and *Bartonella* diversification associated with this host species. Finally, a higher dispersal range may lead to wood mice encountering a wider variety of different host taxa, which are likely to harbour their own *Bartonella* parasites. Field voles (*Microtus agrestis*) and common shrews (*Sorex araneus*) for example are hosts to several *Bartonella* parasites

also detected in wood mice and bank voles (Telfer *et al*., 2007a; Bray *et al*., 2007), so contact with infectious field vole- or shrew-associated fleas may contribute to the variety of *Bartonella* infections found in wood mice.

It is interesting to note that *B. birtlesii* infections were absent in bank voles at one of the current study sites (Rode Hall), despite being present in sympatric wood mice, and found at high prevalence in bank voles at both other sites (Manor Wood and Maresfield & Gordale). A similar pattern was found in a study of Irish rodent populations (Telfer *et al*., 2005). Other previous studies have typically found a higher prevalence of *B. birtlesii* in bank voles compared to wood mice (Telfer *et al*., 2007a; Chapter 2), but results here suggest that persistence of this parasite is clearly not dependent on transmission from bank voles. Indeed, the majority of *B. birtlesii* infections in wood mice and bank voles here were clearly differentiated when characterised at the pITS region.

The richness and diversity of *Bartonella* variants were found to vary geographically. While overall assemblages were relatively similar across all three woodland sites, richness and diversity were generally lower at Rode Hall compared to Manor Wood and Maresfield & Gordale. A study of the genetic variation of *B. grahamii* populations in central Sweden found that rodents sampled from geographically isolated populations were infected with fewer genetic variants compared to populations that were not geographically isolated from other populations, due to mixing between hosts and vectors within a limited spatial scale (Berglund *et al*., 2010b). The sampling grids at Rode Hall were situated within discrete woodland patches surrounded by farmland, and were therefore relatively isolated in comparison to the sampling grids at Manor Wood and Rode Hall, which were within more continuous stretches of woodland. Hence, similar processes to those described by Berglund *et al*. (2010b) may underlie the spatial patterns observed here. Alternatively, differences at Rode Hall may reflect differences in the wider community structure of potential host species at the three sites; there may be a greater diversity of other unsampled host species at Manor Wood and Marefield & Gordale compared to Rode Hall, which contribute to the pool of variants infecting wood mice and bank voles. Further sampling of different potential host species (e.g. field voles or shrews), and characterisation of their *Bartonella* infections, would help to address this knowledge gap.

Molecular studies of parasite populations have been used to infer the occurrence and direction of transmission between host species within multi-host communities (e.g. Biek *et al*., 2012; Streicker *et al*., 2010), and may also reveal the occurrence and direction of historical host-switching events depending on the phylogenetic relationships between variants of a parasite infecting different host species (Antonovics *et al*., 2002; Le Gac *et al*., 2006). Here I attempted to identify the occurrence and direction of host-switching events between wood mice and bank voles for *B. grahamii, B. taylorii* and *B. birtlesii*, by estimating phylogenetic relationships between the pITS variants of each species respectively. However, the relationships were often poorly resolved and it was therefore difficult to infer details of any past host-switching events between wood mice and bank voles for any of these *Bartonella* species. Difficulties in inferring evolutionary relationships between *Bartonella* variants may have arisen due to the genetic marker used. It has previously been suggested that the 16S-23S ITS region may be an inappropriate marker to infer evolutionary relationships between *Bartonella* species, as indels in the aligned sequences of different species can lead to ambiguity of inferred phylogenetic relationships (Ogden & Rosenberg, 2006). However, phylogenetic relationships here were estimated between variants of each *Bartonella* species separately, rather than between species, and as such there were relatively few indels between aligned sequences. Furthermore, while indels were not directly included in the estimation of phylogenetic relationships here, the occurrence of indels was indicated on the resulting phylogenies and broadly match the signals inferred from substitutional changes alone, suggesting that their presence may not have been problematic here.

Instead, evolutionary relationships between *Bartonella* variants may have been poorly resolved here due to the relatively low degree of variation found within the pITS region. Indeed, other loci, such as the citrate synthase gene (*glt*A), are believed to have greater power than the ITS region in delineating evolutionary relationships between *Bartonella* variants (La Scola *et al*., 2003; Birtles & Raoult, 1996; Norman *et al*., 1995). The use of the ITS region here to characterise *Bartonella* infections is justified, as it allowed an assessment of the diversity underscoring the infection dynamics recognised in previous studies that have employed this established and well-used protocol (Telfer *et al*., 2007a; Telfer *et al*., 2005; Chapter 2). However, it would certainly be insightful to investigate evolutionary relationships between variants following genetic characterisation at other

loci. Further to considering alternative genetic loci, it should be noted that ambiguous evolutionary relationships between variants infecting wood mice and bank voles might be evident because historical host-switching events may have included other host species that were not considered here. Including *Bartonella* variants in phylogenetic analyses that infect other species of host that are often associated with bank voles and wood mice (e.g. field voles, shrews and other small mammals) may help to clarify the evolutionary relationships between variants, and therefore increase our ability to infer the occurrence and direction of past host-switching events.

Increased sampling and/or sequencing effort may have revealed greater richness of *Bartonella* variants in either host species. However, this is unlikely, as sequencing effort was substantial (49% of all positive infections) and an analysis of sequencing effort (the proportion of available samples sequenced) indicated that it was not significantly associated with the number of *Bartonella* pITS variants detected in each host species at each site.

Despite appearing to be multi-host generalists, here I have shown that distinguishable assemblages of *Bartonella* variants are found to infect sympatric populations of wood mice and bank voles. This corroborates patterns of fine-scale *Bartonella* host-specificity found in other rodent communities (Paziewska *et al*., 2012; Kosoy *et al*., 2000), but it is the first time it has been demonstrated in British rodent communities. Importantly, this finding suggests that cross species transmission of these flea-borne parasites between wood mice and bank voles may be less frequent than previously thought, and that many of the parasite species we thought were rodent generalists, are actually exclusively found in single hosts. Further work is necessary to determine the mechanisms driving covert host-specificity of *Bartonella* parasites, and should involve characterisation of flea community composition and associations between fleas, *Bartonella* parasites and host species. This will enable investigation of whether the barriers to between-species transmission are ecological or physiological and whether they occur at the parasite-vector, vector-host or parasite-host interface, and ultimately whether limited between-species transmission is likely to be a widespread phenomenon that should be considered in other host-parasite systems.

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3.5 References

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3.6 Appendix

Table A3.1: Total number of blood samples from wood mice and bank voles at each woodland site that tested positive for each *Bartonella* pITS size group (A-E). Multiple blood samples were screened for *Bartonella* spp. infections from 44% of wood mice and 38% of bank voles across all three sites.

pITS group	Manor Wood		Maresfield & Gordale		Rode Hall	
	WM (*N*=587)	BV (*N*=615)	WM (*N*=496)	BV (*N*=311)	WM (*N*=293)	BV (*N*=298)
A	66	6	52	4	36	2
B	49	146	52	26	7	28
C	148	67	167	19	96	99
D	68	101	124	42	98	4
E	8	48	17	46	28	56

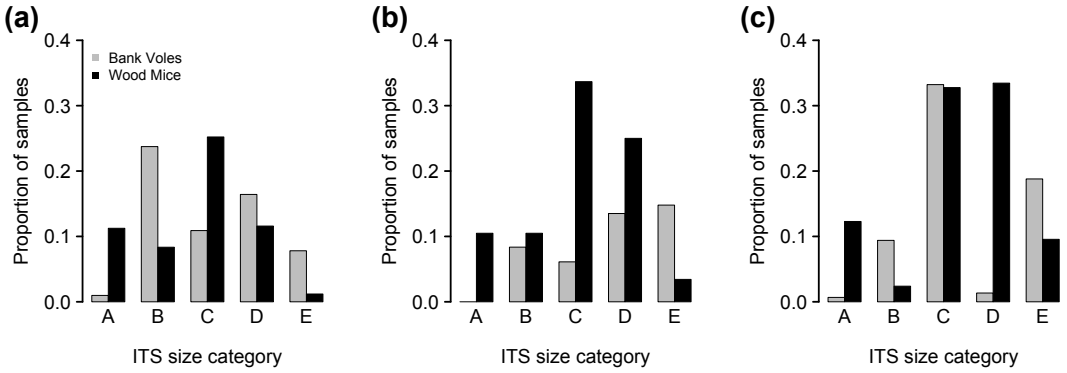

Figure A3.1: The proportion of samples from wood mouse and bank vole individuals at each woodland site that tested positive for each *Bartonella* pITS size group (A-E) (not accounting for potential pseudoreplication due to multiple samples from the same individual). (a) Manor Wood, (b) Maresfield and Gordale, (c) Rode Hall.

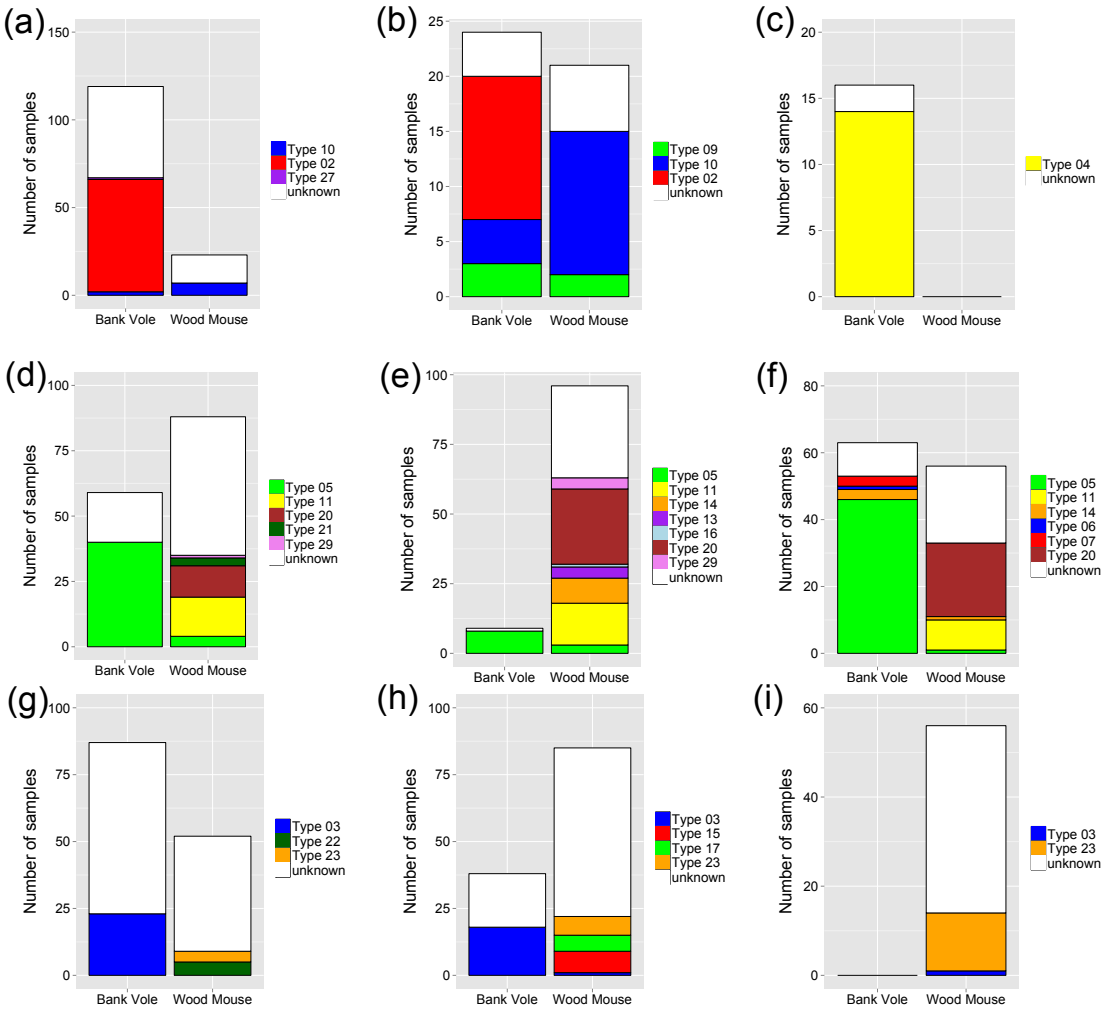

Figure A3.2: The number of each (a-c) *B. grahamii* (d-f) *B. taylorii* and (g-i) *B. birtlesii* variant detected within wood mice and bank voles within each of three woodland sites. Left-hand column = MW, Middle column = MFG, Right-hand column = RH. Colour coding represents different variants within each *Bartonella* species group; separate keys are provided for variants within each *Bartonella* species. Infections that were not sequenced are classed as "unknown" variants. Note that classification of "unknown" variants into their respective *Bartonella* species groups is based on pITS length alone, and therefore may be associated with slight error as outlined in Table 7. Note that the y-axis range varies across *Bartonella* species.

Table A3.2: Variant richness (the number of different variants, *N*) and variant diversity (Shannon-Weiner diversity index, *H*) of a *Bartonella* species detected in wood mice (WM) and bank voles (BV) at all sites combined and at each site individually when variants found on multiple occasions from the same individual were included only once in the data set (414 infections characterised in total). Patterns of *Bartonella* variant richness and diversity across host species and sites are consistent with those found using the entire data set.

***Bartonella* sp.**	**All sites**				**Manor Wood**				**Maresfield & Gordale**				**Rode Hall**			
	N		***H***		***N***		***H***		***N***		***H***		***N***		***H***	
	WM	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**
All	17	12	2.35	1.81	10	7	1.99	1.07	15	6	2.46	1.65	8	7	1.69	1.30
B. doshiae	0	1	NA	0	0	1	NA	0	0	0	NA	NA	0	1	NA	0
B. doshiae-like	1	0	0	NA	1	0	0	NA	1	0	0	NA	1	0	0	NA
B. grahamii	2	5	0.33	0.98	1	3	0	0.31	2	3	0.43	0.91	0	1	NA	0
B. taylorii	8	4	1.47	0.44	5	1	1.26	0	7	1	1.63	0	4	4	0.85	0.59
B. birtlesii	5	1	1.28	0	2	1	0.69	0	4	1	1.22	0	2	0	0.26	NA
B. rudakovii	0	1	NA	0	0	1	NA	0	0	1	NA	0	0	1	NA	0
BGA	1	0	0	NA	1	0	0	NA	1	0	0	NA	1	0	0	NA

Table A3.3: Results of a linear discriminant analysis (LDA) using all data (including multiple identical variants from the same individual) and using host species as the LDA grouping factor. Percentage successful prediction of host species is given for leave-one-out cross validation, a test using 75% of real data as a training set for prediction of the remaining 25%, and a test using 75% of randomised data as a training set for prediction of the remaining 25%. The difference between the successes of the latter two tests was assessed by a chi-squared analysis. An LDA was performed using variants of all *Bartonella* species together, variants of shared *Bartonella* species only (*B. grahamii, B. taylorii* and *B. birtlesii*) and for variants of each shared *Bartonella* species independently. Sample sizes (*n*) are given in each case. Validation tests could not be performed for *B. grahamii* or *B. birtlesii* due to a lack of variation within the assemblages of each host species.

Data used	Test	% Prediction success
All *Bartonella* species	Leave-one-out	96.3
$n = 775$	75:25 training (real data)	98.0 (95%CI: 97.9 – 98.1)
	75:25 training (random data)	72.5 (95%CI: 71.4 – 73.6)
	χ^2 real vs. random	$\chi^2 = 258$, df =1, $p < 0.001$
Shared *Bartonella* species	Leave-one-out	95.3
$n = 616$	75:25 training (real data)	97.5 (95%CI: 97.3 – 97.6)
	75:25 training (random data)	68.0 (95%CI: 66.6 – 69.4)
	χ^2 real vs. random	$\chi^2 = 305$, df =1, $p < 0.001$
B. grahamii	Leave-one-out	92.7
$n = 118$	75:25 training (real data)	NA
	75:25 training (random data)	NA
	χ^2 real vs. random	NA
B. taylorii	Leave-one-out	94.9
$n = 215$	75:25 training (real data)	94.4 (95%CI: 94.1 – 94.7)
	75:25 training (random data)	39.1 (95%CI: 37.2 – 41.1)
	χ^2 real vs. random	$\chi^2 = 689$, df =1, $p < 0.001$
B. birtlesii	Leave-one-out	97.5
$n = 81$	75:25 training (real data)	NA
	75:25 training (random data)	NA
	χ^2 real vs. random	NA

Table A3.4: Results of a linear discriminant analysis (LDA) using a reduced data set that includes identical variants from the same individual only once and using host species as the LDA grouping factor. Percentage successful prediction of host species is given for leave-one-out cross validation, a test using 75% of real data as a training set for prediction of the remaining 25%, and a test using 75% of randomised data as a training set for prediction of the remaining 25%. The difference between the successes of the latter two tests was assessed by a chi-squared analysis. An LDA was performed using variants of all *Bartonella* species together, variants of shared *Bartonella* species only (*B. grahamii, B. taylorii* and *B. birtlesii*) and for variants of each shared *Bartonella* species independently. Sample sizes (*n*) are given in each case. Validation tests could not be performed for *B. birtlesii* due to a lack of variation within the assemblages of each host species.

Data used	Test	% Prediction success
All *Bartonella* species	Leave-one-out	95.0
$n = 380$	75:25 training (real data)	96.7 (95%CI: 96.5 – 96.8)
	75:25 training (random data)	62.5 (95%CI: 61.1 – 63.9)
	χ^2 real vs. random	$\chi^2 = 360$, df =1, $p < 0.001$
Shared *Bartonella* species	Leave-one-out	93.9
$n = 310$	75:25 training (real data)	96.1 (95%CI: 95.9 – 96.3)
	75:25 training (random data)	65.7 (95%CI: 64.2 – 67.3)
	χ^2 real vs. random	$\chi^2 = 299$, df =1, $p < 0.001$
B. grahamii	Leave-one-out	93.1
$n = 87$	75:25 training (real data)	85.8 (95%CI: 85.3 – 86.4)
	75:25 training (random data)	59.4 (95%CI: 58.4 – 60.3)
	χ^2 real vs. random	$\chi^2 = 175$, df =1, $p < 0.001$
B. taylorii	Leave-one-out	93.6
$n = 171$	75:25 training (real data)	92.7 (95%CI: 92.4 – 93.0)
	75:25 training (random data)	34.7 (95%CI: 33.4 – 36.1)
	χ^2 real vs. random	$\chi^2 = 727$, df =1, $p < 0.001$
B. birtlesii	Leave-one-out	96.2
$n = 52$	75:25 training (real data)	NA
	75:25 training (random data)	NA
	χ^2 real vs. random	NA

Table A3.5: Results of a linear discriminant analysis (LDA) using all data (including multiple identical variants from the same individual) and using location as the LDA grouping factor. Percentage successful prediction of host species is given for leave-one-out cross validation, a test using 75% of real data as a training set for prediction of the remaining 25%, and a test using 75% of randomised data as a training set for prediction of the remaining 25%. The difference between the successes of the latter two tests was assessed by a chi-squared analysis. An LDA was performed using variants of all *Bartonella* species together, variants of shared *Bartonella* species only (*B. grahamii, B. taylorii* and *B. birtlesii*) and for variants of each shared *Bartonella* species independently. Sample sizes (*n*) are given in each case. Validation tests could not be performed for *B. grahamii* due to a lack of variation within the assemblages at each site.

Data used	Test	% Prediction success
All *Bartonella* species	Leave-one-out	57.8
$n = 775$	75:25 training (real data)	80.2 (95%CI: 79.6 – 80.8)
	75:25 training (random data)	44.7 (95%CI: 43.3 – 46.1)
	χ^2 real vs. random	$\chi^2 = 269$, df =1, $p < 0.001$
Shared *Bartonella* species	Leave-one-out	60.6
$n = 616$	75:25 training (real data)	74.3 (95%CI: 73.6 – 75.0)
	75:25 training (random data)	42.9 (95%CI: 41.4 – 44.3)
	χ^2 real vs. random	$\chi^2 = 203$, df =1, $p < 0.001$
B. grahamii	Leave-one-out	47.1
$n = 118$	75:25 training (real data)	NA
	75:25 training (random data)	NA
	χ^2 real vs. random	NA
B. taylorii	Leave-one-out	45.6
$n = 215$	75:25 training (real data)	36.5 (95%CI: 34.9 – 38.1)
	75:25 training (random data)	25.7 (95%CI: 23.8 – 27.7)
	χ^2 real vs. random	$\chi^2 = 27$, df =1, $p < 0.001$
B. birtlesii	Leave-one-out	61.7
$n = 81$	75:25 training (real data)	32.0 (95%CI: 30.8 – 33.2)
	75:25 training (random data)	23.4 (95%CI: 21.4 – 25.3)
	χ^2 real vs. random	$\chi^2 = 19$, df =1, $p < 0.001$

Table A3.6: Results of a linear discriminant analysis (LDA) using a reduced data set that includes identical variants from the same individual only once and using location as the LDA grouping factor. Percentage successful prediction of host species is given for leave-one-out cross validation, a test using 75% of real data as a training set for prediction of the remaining 25%, and a test using 75% of randomised data as a training set for prediction of the remaining 25%. The difference between the successes of the latter two tests was assessed by a chi-squared analysis. An LDA was performed using variants of all *Bartonella* species together, variants of shared *Bartonella* species only (*B. grahamii, B. taylorii* and *B. birtlesii*) and for variants of each shared *Bartonella* species independently. Sample sizes (*n*) are given in each case. Validation tests could not be performed for *B. grahamii* or B. *birtlesii* due to a lack of variation within the assemblages of each location.

Data used	Test	% Prediction success
All *Bartonella* species	Leave-one-out	58.2
$n = 380$	75:25 training (real data)	72.7 (95%CI: 71.9 – 73.5)
	75:25 training (random data)	60.1 (95%CI: 58.9 – 61.3)
	χ^2 real vs. random	$\chi^2 = 36$, df =1, $p < 0.001$
Shared *Bartonella* species	Leave-one-out	60.3
$n = 310$	75:25 training (real data)	65.5 (95%CI: 67.6 – 69.5)
	75:25 training (random data)	58.1 (95%CI: 56.7 – 59.5)
	χ^2 real vs. random	$\chi^2 = 12$, df =1, $p < 0.001$
B. grahamii	Leave-one-out	47.6
$n = 87$	75:25 training (real data)	NA
	75:25 training (random data)	NA
	χ^2 real vs. random	NA
B. taylorii	Leave-one-out	49.1
$n = 171$	75:25 training (real data)	46.1 (95%CI: 44.7 – 47.4)
	75:25 training (random data)	18.8 (95%CI: 16.9 – 20.8)
	χ^2 real vs. random	$\chi^2 = 170$, df =1, $p < 0.001$
B. birtlesii	Leave-one-out	73.1
$n = 52$	75:25 training (real data)	NA
	75:25 training (random data)	NA
	χ^2 real vs. random	NA

Chapter 4

The role of fleas in mediating between-species transmission of *Bartonella* spp. in UK woodland rodent communities.

4.1 Introduction

Identifying the extent to which parasites are transmitted between sympatric host species in natural communities is challenging, but crucial for determining how multi-host parasites persist within host communities (Haydon *et al*., 2002; Holt *et al*., 2003; Dobson, 2004; Fenton & Pedersen 2005) and how to target control programmes to reduce the risk of infection (e.g. Rudge *et al*., 2013). In fact, while many parasites are able to infect multiple host species (Cleaveland *et al*., 2001; Woolhouse *et al*. 2001; Pedersen *et al*., 2005), between-species transmission in nature may be rare, resulting in parasites being heterogeneously distributed across species within the host community (Poulin, 2007). Understanding the factors that determine transmission within and between host species is therefore important, as changes to community composition (e.g. species loss or gain) or to interactions between existing hosts imposed by environmental or control-related perturbations may result in increased infection risk, and even emergence of infection into novel host species, depending on these underlying factors (Kilpatrick *et al*., 2006b; Woodroffe *et al*., 2009; Allan *et al*., 2010; Simpson *et al*., 2012).

Factors that can determine the extent of parasite transmission between different host species in a community can be broadly categorised into two conceptual "filters": host-parasite encounter barriers and host-parasite incompatibility (Combes, 2005; Poulin, 2007) (Figure 4.1a-c). An encounter barrier implies that while a parasite has the physiological capability to infect a particular host species, the ecological opportunity for transmission does not exist. For example, experimental infections have shown that many parasites are capable of infecting hosts outside of their observed host range (Reed & Hafner, 1997; Perlman & Jaenike, 2003; Munoz-Antoli *et al*., 2006; King & Cable,

2007), suggesting that low host-parasite encounter rates may contribute to the absence of infections in these hosts in nature.

In contrast, host-parasite incompatibility may render a parasite unable to establish infection in certain host species, even if opportunities for transmission are common (Figure 4.1c). Intuitively, there is great variation in the compatibility of a parasite species to infect a range of host species, and this phenomenon has been demonstrated experimentally in several systems (Tompkins & Clayton, 1999; Kosoy *et al*., 2000; Komar *et al*., 2003; Esberard *et al*., 2005; Gilbert & Webb, 2007; Palinauskas *et al*., 2008), with many parasites unable to establish infection or suffering markedly lower fitness when introduced to new host species. Such incompatibility may arise due to tight co-evolution of a parasite lineage with particular host species, such that certain parasite genotypes can only infect certain host genotypes. Indeed, phylogenetic signals of host compatibility are often evident, which supports this notion, such that a parasite may be compatible with several closely related host species, which are more likely to share traits to which a parasite can simultaneously adapt, but not with more distantly related species (Medeiros *et al.,* 2013; Davies & Pedersen 2008, Gilbert & Webb, 2007; Perlman & Jaenike, 2003; Krasnov *et al*., 2004b).

Understanding whether host-parasite encounter rate or compatibility (or both) determine whether a parasite transmits between host species is important for future predictions of disease spread. Environmental changes that alter interactions within a community will have different consequences for emergence into novel host species, and persistence of the parasite in the community as a whole, depending on the underlying filters limiting parasite transmission. If transmission is primarily restricted due to lack of encounters, then rapid emergence into a new host may occur if encounters become likely (Reullier *et al*., 2006; Richomme *et al*., 2006). If transmission is restricted to due a lack of host-parasite compatibility, however, then emergence into currently uninfected host species may be less likely, or may take more time as new, more compatible strains of the parasite evolve as a result of more regular encounters with new host species (Antia *et al*., 2003; Lloyd-Smith *et al*., 2009). Discerning the relative importance of encounter versus compatibility filters in nature is challenging, however, as the rate of host-parasite

encounters may be subject to fine-scale influences beyond hosts and parasites simply existing in sympatry (Kuris *et al*., 2007).

For microparasites (e.g. protozoa, bacteria, viruses) transmitted by vectors such as haematophagous (blood-feeding) ectoparasites, the rate of encounter with hosts, and therefore transmission between individuals, is influenced by the abundance and distribution of the vectors. This introduces further complexity to multi-host systems, as the vectors, being parasites themselves, will also have variability in the potential host species they infect, as a result of their own encounter/compatibility filters (e.g. Tompkins & Clayton, 1999; Hassan *et al*., 2003; Krasnov *et al*., 2004a; Kilpatrick *et al*., 2006; Jones *et al*., 2007; Hamer *et al*., 2009). Transmission of a microparasite between different, sympatric host species may be restricted, even if it is compatible with both, because the necessary vector does not feed on all host species (Figure 4.1a). Such an encounter barrier may be lessened, if the parasite can be vectored by several co-occurring species of ectoparasite of which at least one feeds on multiple host species (Kimura *et al*., 2010; Njabo *et al*., 2011).

Understanding patterns of between species transmission can be more difficult if ecological differences between host species preclude the movement of vectors between them. Therefore, even if an ectoparasite appears to utilise multiple different host species equally, discrete populations may actually be associated with each host species ('covert host specialism') (Figure 4.1b). However, ectoparasites comprise a diversity of different taxa, which differ in their ecology (e.g., dispersal ability/motility, time spent on host, propensity to switch between hosts) and in their performance as vectors (Randolph, 1998). Such encounter barriers, mediated largely by the activity of host species, are perhaps less likely where the behaviours of vector species are not completely dependent on their hosts, such is the case with many dipteran ectoparasites (e.g. mosquitoes, Hamer *et al*., 2014, but see also Harrington *et al*., 2005). However, for vectors with limited mobility, which therefore rely on more opportunistic host location strategies, such as fleas (Marshall, 1981; Krasnov, 2008), transfer between individuals of different host species may require them to be in close proximity, or use the same habitat space, for a sufficient period of time (Krasnov & Khokhlova, 2001).

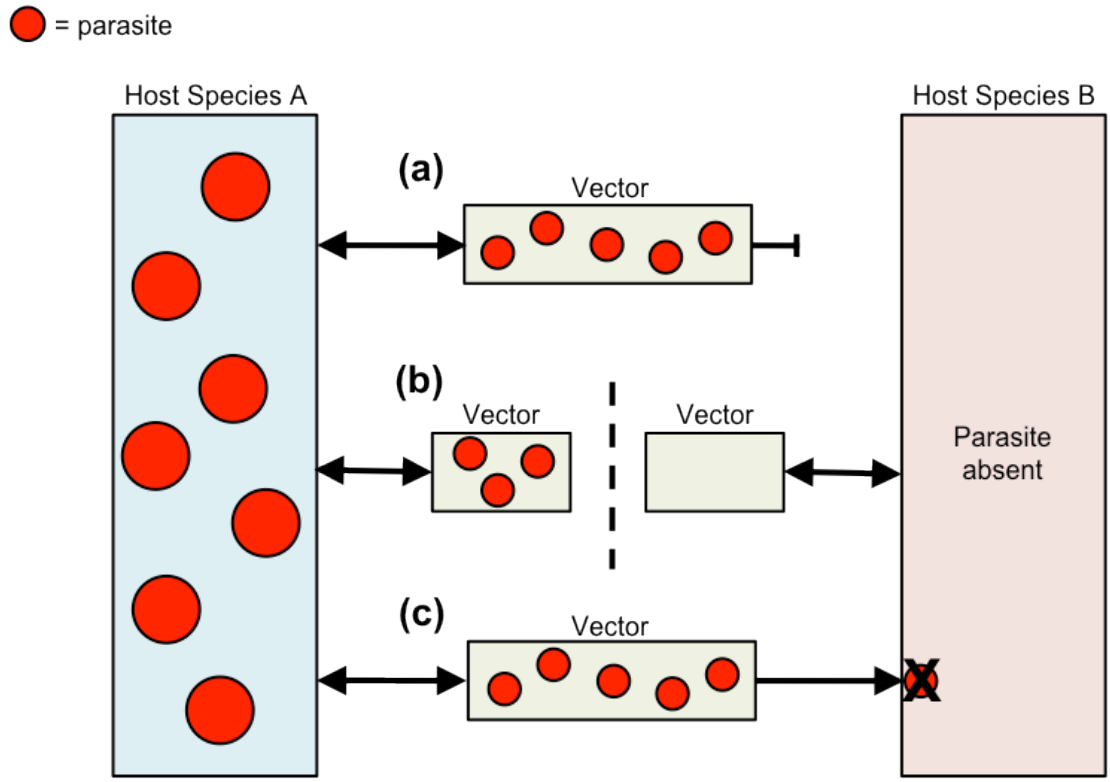

Figure 4.1: For transmission of a parasite vectored by a haematophagous ectoparasite to occur between two different host species (Host A and Host B), an individual vector must be able to feed on and pick up the pathogen from an individual of one host species, maintain the pathogen, feed on the other host species and for that infection to establish in the new host. As such, between-species transmission of a vector-borne parasite between host species may be less likely if: (a) the vector is adapted to only one of the host species, (b) the vector is adapted to both host species, but separate isolated populations infest each, such that the ecological opportunity for transfer of infectious vectors between individuals of different host species does not arise, (c) the vector is adapted to both host species and is ecologically capable of transferring between individuals of different host species, but the microparasite is not compatible with both host species.

Bartonella parasites that infect woodland rodents offer an opportunity to investigate the role of ectoparasitic vectors in mediating between-species transmission within a multi-host community. These are gram-negative haemoparasites that parasitise a diverse range of mammalian hosts (Breitschwerdt & Kordick, 2000; Kosoy *et al*., 2012) and are particularly well studied in wild rodents (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al*., 2007a; Knap *et al*., 2007; Bray *et al*., 2007; Gil *et al*., 2010; Welc-Faleçiak *et al.,* 2010; Paziewska *et al*, 2012). Transmission between individuals occurs via the feeding activity of blood-feeding flea vectors (Bown *et al*., 2004; Morick *et al*., 2011).

In the UK, several species of *Bartonella* are endemic within, often sympatric, populations of wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*). Some *Bartonella* parasite species are strongly associated with one or other of these common rodent species, and there is a debate about the role that between species transmission plays for parasite persistence (Birtles *et al*., 2001; Telfer *et al.,* 2007a; Chapter 2). Several other species of *Bartonella* are reported to infect both wood mice and bank voles in sympatry (Birtles *et al*., 2001; Telfer *et al.,* 2007a; Chapter 2), however, recent work has established that these apparently generalist species actually comprise a complex of genetic variants (defined by the 16S-23S rRNA pITS region; Chapter 3). This analysis changed our view of the level of host sharing between the seemingly generalist *Bartonella* species; and in fact I found that variants of the same *Bartonella* species had different distributions among host species, with some being exclusively found in wood mice, some in bank voles, and only a minority was found to infect both. Overall, the assemblages of *Bartonella* variants found in wood mice and bank voles differed significantly (Chapter 3), suggesting that transmission of even apparent generalist parasites between these two host species is less common than initially suspected.

This apparently limited *Bartonella* transmission between sympatric wood mice and bank voles must result from either (i) incompatibility between certain *Bartonella* variants and host species (a compatibility filter; Figure 4.1c), (ii) a barrier that inhibits encounters between certain *Bartonella* variants and host species (an encounter filter; Figure 4.1a or 4.1b), or both. Without reciprocal infection experiments, incompatibility between variants and host species is difficult to confirm, but has been demonstrated for *Bartonella* parasites in a different rodent community (Kosoy *et al*., 2000). However, the

potential for host-*Bartonella* encounter barriers may be assessed by an investigation of the vector capacity and host preferences of the prevailing flea community.

The same flea species have been found to infect sympatric wood mice and bank voles in the UK (Noyes *et al*., 2002) and Ireland (Telfer *et al*., 2005), suggesting that specific relationships between fleas and hosts (Figure 4.1a) may not create an encounter barrier. However, an assessment of host preferences for these different flea species is lacking. Heterogeneous distributions of flea species, suggesting preferential feeding of different flea species on different host species, have indeed been identified in several other rodent communities across the globe (e.g. Krasnov *et al*., 2003; Khokhlova *et al*., 2012), and such host preferences here may be relevant to the dynamics of *Bartonella* spp. transmission if heterogeneity is extreme. Furthermore, flea-mediated encounter barriers can only be assessed with knowledge of the relationships between flea species and *Bartonella* parasites. If a parasite is vectored by multiple flea species, of which at least one feeds on both wood mice and bank voles, then host-parasite encounters are less likely to be restricted. Relationships between flea species and *Bartonella* parasites have been investigated elsewhere (Abbot *et al*., 2007; Brinkerhoff *et al*., 2010; Morick *et al*., 2010), and strictly specific relationships have been largely refuted. However, such relationships have not yet been investigated in any detail in rodent-*Bartonella* systems in the UK.

In the absence of associations between flea species, *Bartonella* parasites and host species that may restrict *Bartonella* transmission between wood mice and bank voles, ecological differences between these two host species may still inhibit the transfer of infectious fleas between them, and therefore limit the opportunity for *Bartonella* spp. transmission (Figure 4.1b). Wood mice and bank voles often show interspecific segregation at the microhabitat scale (Bergstadt, 1965; Geuse, 1985), and differ in their activity patterns (nocturnal wood mice versus crespuscular bank voles; Greenwood, 1978), extent of spatial movement (larger home range for wood mice compared to bank voles; Crawley, 1969; Carslake *et al*., 2005) and in their diet and habitat vegetation preferences (largely granivorous wood mice compared to folivorous/granivorous bank voles; Watts, 1968; Canova, 1993), and these differences may reduce or even, preclude contact. Transfer of individual fleas between wood mice and bank voles has been documented in the UK (Noyes *et al*., 2002) and Ireland (Telfer *et al*., 2005), but

multiple species of flea were considered collectively. Here, I assess interspecific flea transfer within this wild rodent system to assess whether different species of flea are equally likely to transfer between wood mice and bank voles.

To identify the potential for flea-mediated encounter barriers to the transmission of *Bartonella* parasites between wood mice and bank voles in the UK, I addressed the following questions:

1. What species of flea are found to infect sympatric populations of wood mice and bank voles across woodland sites in northwest England?
2. Are certain flea species more often found on one host species compared to the other, suggesting heterogeneous host preferences?
3. Which *Bartonella* parasites (pITS variant) are found within different flea species?
4. Are *Bartonella* parasites found exclusively to infect a single host species associated with certain flea species that display a specificity/preference for that same host?
5. In the absence of any host-flea-*Bartonella* associations, is there evidence to suggest that different subpopulations of the same flea species circulate within the wood mouse and bank vole populations?

4.2 Methods

4.2.1 Resolving host-*Bartonella* relationships

The community of *Bartonella* parasites found to infect wood mice and bank voles in natural woodland habitats in northwest England was described in Chapter 3. Rodents were captured and blood sampled at three different woodland sites: Manor Wood (MW; N 53.3301°, E -3.0516°), Maresfield & Gordale woods (MFG; N 53.2729°, E -3.0615°) and Rode Hall (RH; N 53.1213°, E -2.2798°). Samples were collected from MW in 2011, and from MFG and RH in 2012. Blood samples were screened for *Bartonella* infections, and many infections were genetically characterised at a partial fragment of the 16S-23S internal transcribed spacer (pITS) region, and identified to species based on a BLAST search in GenBank. Full details of field methodology and *Bartonella* diagnostics are given in Chapter 3. The distribution of *Bartonella* pITS variants across host species was assessed, and variants were classed as host-exclusive (variants that were only ever found to infect one host species) or host-shared (variants that were found to infect both species at least once).

4.2.2 Rodent flea sampling

Fleas were collected from wood mice and bank voles during 2012 and 2013, as part of an ongoing longitudinal study of rodent parasite infection dynamics, to identify the assemblage of flea species found to infest this rodent community. Full details of general field methodology are provided elsewhere (Chapter 2). Sherman live-traps (Alana Ecology) were set each month from May to December in both years within three areas of mixed deciduous woodland in northwest England. Two of these sites were used to determine the distribution of *Bartonella* parasites across rodent host species in 2012 (MFG and RH; see Section 3.1), and a third site, Haddon Wood (HW; N 53.2709°, E -3.0268°), was used just for flea assemblage studies. At all three sites, captured animals were given unique subcutaneous PIT-tags to enable future identification, and blood samples were taken each month to screen for active *Bartonella* infections. Fleas were

removed from individuals by brushing the fur over a water bath, then collected from the water and stored individually in Eppendorf tubes containing 90% ethanol. Fleas were subsequently identified morphologically to species, using a binocular light microscope and taxonomic key (Whitaker, 2007).

4.2.3 Resolving flea-host relationships

The capability of each flea species to use each rodent species as a host was assessed by comparing the assemblages of fleas occurring on wood mice and bank voles, across all months, years and woodland sites. I make the simplifying assumption that the presence of a flea on an individual rodent indicates the suitability of that rodent as a blood-meal source, and conclude that if a given flea species was found to infect individuals of both host species, then it is capable of feeding on both species as a host given the opportunity (i.e. true host-generalist). Conversely, if a flea species was only ever detected on individuals of just one host species, then we conclude that the ability to infest both host species is not evident (i.e. host-exclusive). Note that because flea species were often represented by a very small number of specimens, we could not confidently assume that they were strictly incapable of infecting both host species in all cases. The diversities of the flea assemblages found to infect each host species were compared by calculating Shannon-Weiner diversity indices. Rather than absolute numbers of fleas collected from each host species (which may include multiple fleas of the same species from the same individual), occurrence was measured as presence/absence of a flea species per host individual on at least one occasion.

It is possible that even if a species of flea is able to infest both host species, one host may have a tendency to be infected by a given flea species more than the other (i.e. it displays a 'host-preference' rather than being host-exclusive). Flea mediated encounter barriers to between-species transmission of *Bartonella* spp. may therefore still emerge due to heterogeneous host preferences, and so I assessed whether such patterns exist here. For each flea species, Chi-squared tests were used to assess whether they infected wood mice and bank voles in equal proportions (i.e. true host-generalist), or whether a greater proportion of infestations were found on one host species compared to the other

(i.e. host preference). To avoid problems associated with multiple sampling of some individuals, an individual was counted as infected if at least one flea of that species was collected from it on at least one occasion. Care should be taken with the interpretation of these patterns however; flea sampling was, in part, ad-hoc, so we do not always know the total numbers of each host species sampled and therefore how many individuals were not infected with any fleas. Therefore proportions infected are calculated as the number of individuals infected with a given flea species out of the total number of individuals infected with any flea.

4.2.4 Detection of *Bartonella* parasites within individual fleas

DNA was extracted from individual fleas using a Promega Wizard® Genomic DNA Purification Kit (Promega Corporation, USA). Fleas were first washed in ultrapure water to remove excess ethanol and then crushed in an Eppendorf tube using a micro-pestle. 200μL chilled Nuclei Lysis Solution was added to the tube. The solution was vortexed for 10 seconds and then incubated at 65°C for 30 minutes. After cooling to room temperature, 66μL Protein Precipitation Solution was added. The mixture was briefly vortexed before chilling on ice for 5 minutes, followed by centrifugation at 14000xG for 4 minutes to precipitate the protein. The DNA-containing supernatent was transferred to a fresh Eppendorf tube containing 200 μL 100% isopropanol, and mixed gently by inversion. The mixture was centrifuged at 14000xG for 1 minute to precipitate the DNA pellet, which was washed in 70% ethanol before re-suspension in 10μL TE buffer.

DNA extractions were quantified using the Qubit™ double-stranded DNA high-sensitivity assay (Invitrogen, UK), according to the manufacturer's guidelines. As DNA was extracted from individual fleas, the resulting quantity of DNA was often very low. Therefore, to further establish extraction success, each extraction was used as a template in a PCR targeting the conserved invertebrate 18S rRNA gene. 1μL DNA template was incorporated into 10μL PCR reactions containing 5μL Biomix Red PCR Readymix (Bioline), 3.6 μL ultrapure water and 0.5μL each of 10μM forward and reverse primers (18SF: CTGGTTGATYCTGCCAGT; 18SR: TCTCAGGCTCCYTCTCCGG;

corresponding to primers '1' and '6' in Hendriks *et al*., 1991). Reaction mixtures were exposed to a thermal programme consisting of 4 minutes initial denaturation at 95°C followed by 35x[95°C for 15s, 60°C for 15s, 72°C for 45s], with a final extension of 72°C for 7 mins. Positive products (~370 bp) were confirmed by visualization on 1.5%(w/v) agarose gels stained with Ethidium Bromide, run for 30 minutes at 120V.

To determine whether individual fleas were carrying *Bartonella* parasites, flea-extracted DNA was used as a template in *Bartonella* genus-specific PCR assays. A 300-500bp fragment of the *Bartonella* 16S-23S internal transcribed spacer (ITS) region was targeted using the primers of Telfer *et al*. (2005). Full details of the PCR protocol, including primer sequences, reaction conditions and thermal cycling programmes, are given in Chapter 2, Section 2.2.2. The partial ITS (pITS) region of DNA targeted by this PCR assay varies in length between different species of *Bartonella* (Roux & Raoult, 1995; Birtles *et al*., 2000; Houpikian & Raoult, 2001), and species-level identity of any positive infections were therefore first determined by assessing the size of PCR amplification products (as in Telfer *et al*., 2005, Chapter 2 & 3).In addition, given the great variation in variants, the PCR products of singly-infected with *Bartonella* species were sequenced, according to the methodology presented in Chapter 3, Section 3.2.2.2, to determine the diversity of pITS *Bartonella* variants infecting each flea species. All pITS sequences were identified to species according to BLAST searches within GenBank (see Chapter 3 for details).

4.2.5 Resolving flea-*Bartonella* relationships

To assess whether flea species differed in their vectorial capacity for *Bartonella* parasites in general, the number of *Bartonella*-positive fleas was determined for each flea species. For fleas with at least one positive specimen, a generalised linear model (binomial errors, logit link) was used to compare the prevalence of *Bartonella* spp. across flea species. The binomial response variable was the presence/absence of any *Bartonella* parasites, and flea species (a factor with 7 levels) was investigated as the single explanatory variable.

To test whether interactions between specific *Bartonella* variants and flea species play a role in structuring the *Bartonella*-host interactions, I used a Mantel test to evaluate whether pair wise similarities between *Bartonella* variants in terms of the host species they were found on were correlated with pairwise similarities based on the flea species they were carried by. Only variants that were detected in host and flea samples were used in this analysis (N=16 variants). Similarities were based on the Morisita-Horn index, and significance of associations between these two similarity matrices was based on 10,000 randomised permutations, following the methods of Medeiros *et al*. (2013). All analyses were performed in the *vegan* package of R v2.14.2.

A Monte Carlo approach was then used to assess whether there were any associations between specific flea species and *Bartonella* variants. The abundance of each variant detected within each flea species was compared to a simulated distribution of abundance, based on random assignment of variants to flea species within the sample (analogous to the method of Medeiros *et al*., 2013). For 100,000 simulations, all *Bartonella*-positive flea individuals were randomly assigned to *Bartonella* variants based on the proportion of each variant present within the sample. The original number of infections per flea species was maintained in each run of the simulation. The 95% confidence limits for the abundance of each flea species-*Bartonella* variant pair was then calculated. If the actual number of infections of a given *Bartonella* variant within a given flea species fell outside the 95% confidence limits of the simulated distribution, an association between this variant and flea species was accepted. If host-exclusive *Bartonella* variants were positively associated only with flea species that were exclusive to or showed a preference for the same host species, this would support a flea-mediated encounter filter between *Bartonella* variant and host species. If not, and especially if there was a positive association between host-exclusive variants and generalist flea species, the role of flea-host associations in precluding *Bartonella* transmission between wood mice and bank voles would not be supported.

4.2.6 Assessing the potential for an ecological encounter barrier to between-species transmission

In the absence of any specific relationships between host species, flea species and *Bartonella* parasites, barriers to between-species *Bartonella* transmission may be possible if host populations and their associated fleas are segregated in space or habitat use, such that that fleas are not able to transfer between rodent species. To address this possibility, I compared the *Bartonella* parasites found within generalist fleas collected from each rodent species. If host-exclusive *Bartonella* variants were found only in fleas that were collected from the relevant host, this would suggest the possibility of a transmission barrier due to limited flea transfer. The host-specificity of *Bartonella* variants were determined from data collected in 2011 and 2012, whereas fleas were collected from hosts during 2012 and 2013, and used an additional novel site (HW). Care was taken to corroborate patterns found here, using only data for which the characterisation of *Bartonella* infections in rodents and fleas at the same sites and in the same sampling year were available (i.e. MFG and RH in 2012).

4.3 Results

4.3.1 Resolving host-*Bartonella* relationships

A variety of pITS *Bartonella* variants have previously been found to infect wood mice and bank voles (Chapter 3). However, their distribution is heterogeneous across these two rodent hosts, with 14 variants found exclusively in wood mice, six found exclusively in bank voles, and six found in both host species. For clarity, an overview of the relationships between *Bartonella* variants and each host species is shown in Table 4.1.

Table 4.1: An overview of the associations between rodent species (wood mice and bank voles) and the *Bartonella* parasites found to infect them, as described in Chapter 3. Parasites are grouped according to species (*B. doshiae, B. doshiae*-like, *B. grahamii, B. taylorii, B. birtlesii, B. rudakovii* and BGA) on the basis of partial 16S-23S rRNA ITS sequence similarity with previously identified *Bartonella* parasites in GenBank (see Chapter 3). Variants within each *Bartonella* species are differentiated into "Types" according to differences at this region of DNA. Wood-mouse exclusive variants were only ever detected in wood mice, bank vole-exclusive variants were only ever found in bank voles, and host-shared variants were found at least once in both host species.

Bartonella sp.	pITS size group	WM-exclusive	BV-exclusive	Host-shared
B. doshiae	A	-	Type 01	-
B. doshiae-like	A	Type 12		-
B. grahamii	B	Type 27	Type 02 Type 04	Type 09 Type 10
B. taylorii	C	Type 13 Type 16 Type 20 Type 21 Type 29	Type 06 Type 07	Type 05 Type 11 Type 14
B. birtlesii	D	Type 15 Type 17 Type 22 Type 23 Type 25 Type 26	-	Type 03
B. rudakovii	E	-	Type 08	-
BGA	E	Type 24	-	-

4.3.2 Rodent flea assemblages

Fleas were collected from 115 wood mice (158 fleas) and 119 bank voles (179 fleas) across the three woodland sites throughout the study period. Single fleas were collected from the majority of individuals, although multiple fleas were collected from 23% of wood mice and 25% of bank voles (maximum 6 and 8, respectively), either because several fleas were taken on the same sampling occasion or because fleas were collected from the same individual on multiple separate occasions (Figure 4.2). In the majority of cases where multiple fleas were sampled from an individual (57% of wood mice and 57% of bank voles), the fleas collected were all of the same species, although there were occasions where two or three different species were collected, even on the same sampling date (see appendix Table A1 and A2). Seven flea species were identified morphologically: *Amalareus penicilliger mustelae*, *Ctenophthalmus nobilis vulgaris*, *Hystrichopsylla talpae talpae*, *Megabothris turbidus*, *Palaeopsylla sorcis*, *Rhadinopsylla pentacantha* and *Typhlocerus poppei poppei*.

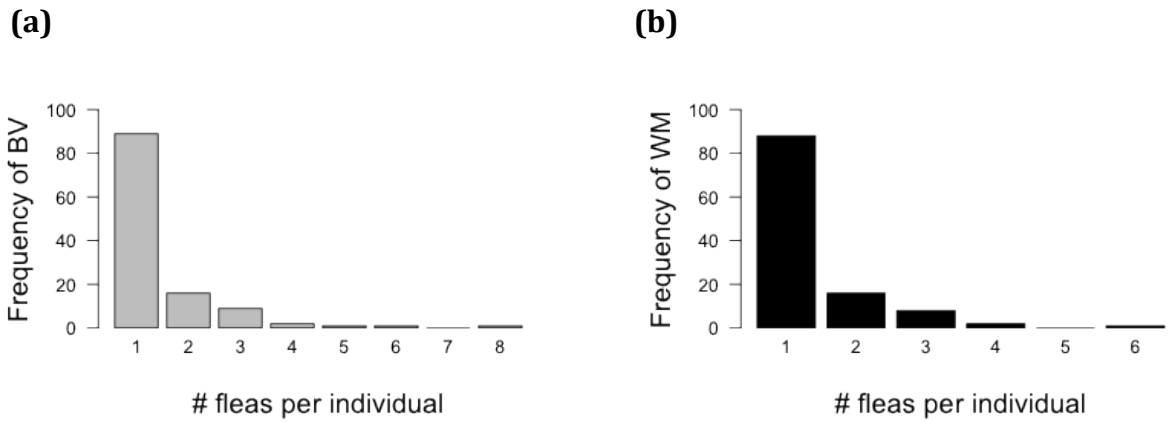

Figure 4.2: Frequency distributions of the number of fleas collected per individual (a) bank vole and (b) wood mouse. The majority of individuals of both host species only provided a single flea.

4.3.3 Relationships between flea and host species

All flea species, except *T. p. poppei*, were found at least once on individuals of both rodent species, even though the numbers of specimens collected in some cases were small (e.g *H. t. talpae*, *M. turbidus* and *P. sorcis*) (Table 4.2). This suggests that both wood mice and bank voles are able to provide a suitable host environment for the majority of flea species detected, and that most fleas are therefore host generalists. *T. p. poppei* was only found on wood mice, meaning evidence that it can use both rodents as a host is lacking. However, with such a small number of specimens collected (n=6), it is not possible to conclude whether this flea is a true host-specialist.

Table 4.2: The numbers of individual bank voles and wood mice that were found to be infested with each of seven flea species, on at least one sampling occasion, and the total number of specimens collected from each rodent species. Note that an individual may have been infested by more than one flea species; therefore the total number of flea-infested wood mice or bank voles is not necessarily the sum of the numbers infested with each flea species.

Flea species	# infected individuals		# specimens collected	
	BV	**WM**	**BV**	**WM**
Ctenophthalmus nobilis vulgaris	77	89	110	121
Amalareus penicilliger mustelae	36	12	40	14
Rhadinopsylla pentacantha	16	5	18	5
Typhlocerus poppei poppei	0	4	0	6
Hystrichopsylla talpae talpae	8	5	9	5
Megabothris turbidus	1	6	1	6
Palaeopsylla sorcis	1	1	1	1

While most flea species appear able to infect both wood mice and bank voles, there were indications of subtle differences between the respective flea assemblages of the two host species. Firstly, the assemblage of fleas infecting wood mice was less diverse than the assemblage associated with bank voles (Shannon-Weiner diversity indices were 0.92 for wood mice and 1.07 for bank voles). There were also some indications that different species of flea infected one rodent species as a host more often than the other.

C. n. vulgaris was the most common flea found on both hosts, however, it was found on a greater proportion of flea-infected wood mice (73%) compared to bank voles (55%; χ^2 = 4.57, d.f.= 1, p= 0.03; Figure 4.3). In contrast, *A. p. mustelae* was found on a higher proportion of flea-infected bank voles (30%) compared to wood mice (10%; χ^2 = 14.09, d.f.= 1, p<0.001; Figure 4.3), and so too was *R. pentacantha* (found on 13% of flea-infested bank voles and 4% of wood mice; χ^2 = 4.864, d.f.= 1, p=0.03, Yates' continuity correction applied; Figure 4.3). Very few specimens of all other flea species were collected; therefore, assessments of potential host-preferences are not possible.

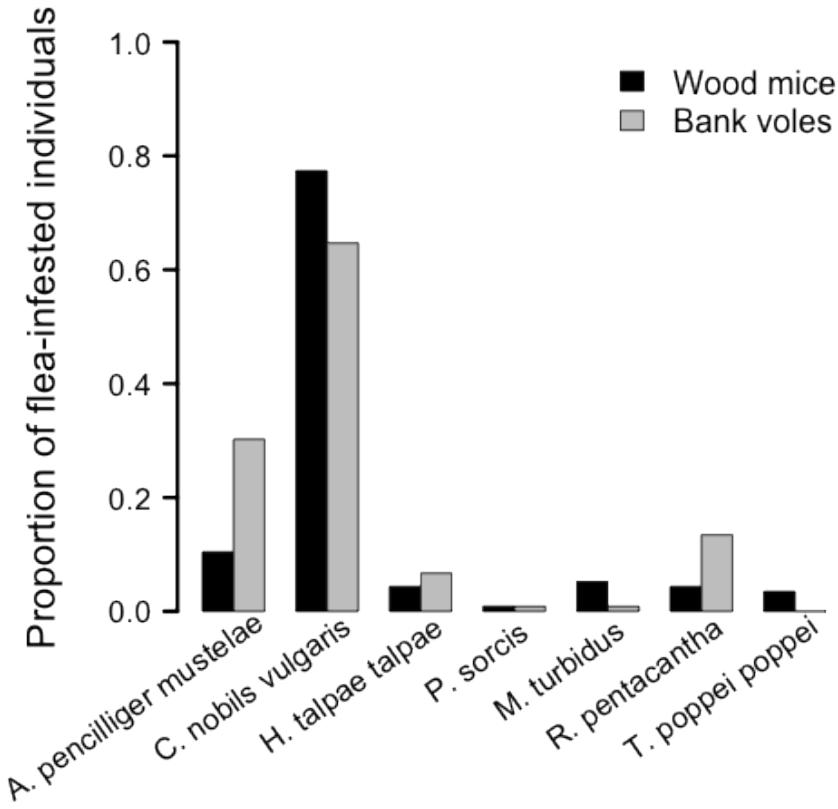

Figure 4.3: The proportion of flea-infested wood mice (black bars) and bank voles (grey bars) infected with at least one of each of seven different species of flea. A significantly greater proportion of flea-infested wood mice were infested with *C. n. vulgaris* than bank voles, and a significantly greater proportion of flea-infested bank voles were infested with *A. p. mustelae* and *R. pentacantha* than wood mice.

4.3.4 Detection and characterisation of *Bartonella* infections within individual fleas

DNA was extracted from 229 individual fleas. The extractions of 39 specimens resulted in no detectable DNA according to the Qubit™ quantification assay, but the PCR targeting the invertebrate 18S rRNA gene produced a clear amplicon in all cases, indicating that DNA was present in all extracts.

Bartonella parasites were detected in 110 fleas (37%) (Table 4.3). Obvious *Bartonella* co-infections (multiple partial ITS amplicons) were detected in 13 fleas, and constituted a range of *Bartonella* species combinations (Appendix Table A4.3). These infections were necessarily omitted from DNA sequence characterisation at the partial ITS region. Nevertheless, *Bartonella* parasites were sequenced at the pITS region from a considerable proportion (50-100%) of PCR-positive specimens of each flea species (Table 4.3).

Table 4.3: Number of fleas collected, number and % of *Bartonella*-positive fleas, and number and % of positive specimens for which *Bartonella* parasites were characterised, for each flea species. Wood-mouse exclusive variants are highlighted in pink; bank-vole exclusive variants are highlighted in green; variants previously detected in both rodents ("shared") are highlighted in purple; variants detected for the first time in this study, and therefore so far only detected in fleas, are highlighted in grey. Numbers of flea specimens that were positive for each *Bartonella* pITS variant are shown in brackets. * indicates when a host-exclusive variant was found in a flea taken from the alternative host species.

Flea species	***A p. mustelae***	***C.n. vulgaris***	***H.t. talpae***	***M. turbidus***	***P. sorcis***	***R. pentacantha***	***T.p. poppei***
No. Specimens	46	210	12	5	1	21	4
No. *Bartonella*-positive	22 (48%)	79 (38%)	4 (33%)	4 (80%)	0 (0%)	1 (5%)	0
No. *Bartonella* parasites characterised genetically	17 (77%)	66 (84%)	2 (50%)	4 (100%)	NA	1 (100%)	NA

	Host sp. from which collected:	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**	**WM**	**BV**		**WM**	**BV**	
Bartonella variants detected	WM exclusive			T12 (3) T13 (3) T20 (2) T21 (1) T23 (3) T24 (8)	T12 (1)* T20 (1)* T24 (1)*	T23 (1)					T23 (1)		
	BV exclusive		T02 (3) T08 (7)	T01 (1)* T02 (1)* T07 (1)*	 T02 (1) T07 (3)			T02 (1)*					
	Host shared	T10 (2) T11 (2)	T05 (1)	T03 (1) T05 (3) T09 (2) T11 (2)	T03 (3) T05 (11) T11 (1) T14 (5)			T05 (1) T09 (1)					
	Fleas only	T28 (1)	T31 (1)	T30 (2) T33 (1) T34 (1)	T30 (3)			T28 (1) T32 (1)					

3.3.5 Resolving flea-*Bartonella* relationships

The prevalence of *Bartonella* spp. varied across flea species (Table 4.3). *Bartonella* parasites were absent from all specimens of *P. sorcis* and *T. p. poppei*, and these species were omitted from the statistical analyses. The GLM found that *Bartonella* spp. prevalence did not differ between *A. p. mustelae*, *C. n. vulgaris*, *M. turbidus* or *H. t. talpae*, but was significantly lower in *R. pentacantha* than in other flea species (Table 4.4; Figure 4.4). Highest prevalence occurred within *M. turbidus* (80%), but the small sample size of this flea resulted in large error associated with predicted prevalence, and this difference was therefore not significant.

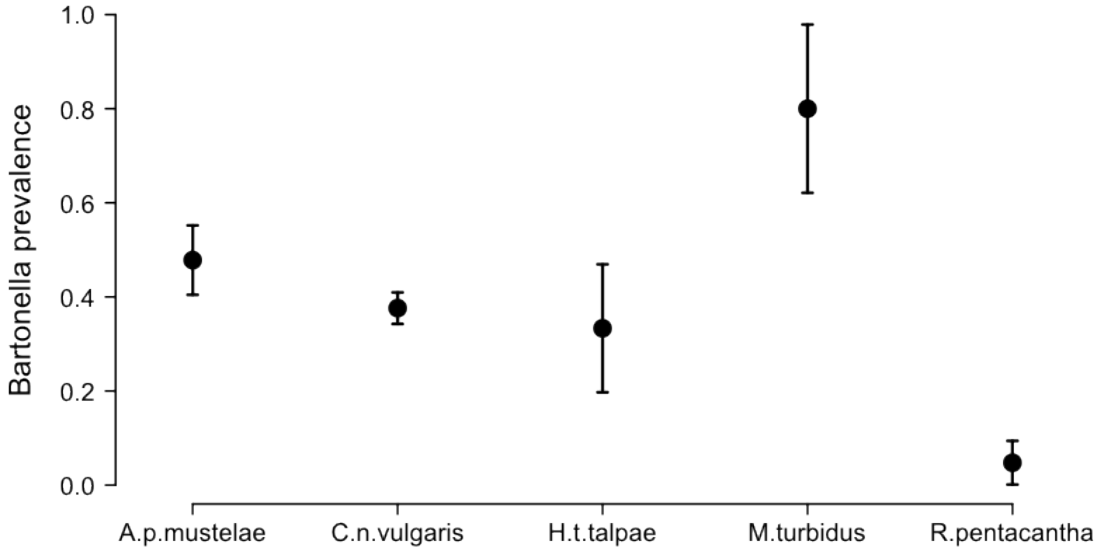

Figure 4.4: Overall *Bartonella* prevalence within samples of the seven different flea species, as predicted from a binomial generalised linear model of infection status per individual flea. Bars are standard errors.

Table 4.4: Parameter estimates for a generalised linear model of overall *Bartonella* prevalence in sampled fleas as a function of flea species. Prevalence was significantly lower in samples of *R. pentacantha*.

Flea species	β	S.E.	*z*	*p*
Intercept (*A. p. mustelae*)	-0.08701	0.29516	-0.295	0.77
C. n. vulgaris	-0.41874	0.32774	-1.278	0.20
H. t. talpae	-0.60614	0.67979	-0.892	0.37
M. turbidus	1.47331	1.15634	1.274	0.20
R. pentacantha	-2.90872	1.06628	-2.728	0.01**

Twenty-two different *Bartonella* pITS variants were detected overall (Table 4.3), representing seven different species according to sequence comparison with known species in GenBank. Of these variants, six have previously only been detected within wood mouse (Types 12, 13, 20, 21, 23 and 24), four have only been detected in bank vole (Types 01, 02, 07 and 08) and six have been found to infect both host species (Types 03, 05, 09, 10, 11, 14) (Chapter 3; Table 3.1). I also detected six new variants within flea samples, including three novel *B. grahamii* variants (Types 28, 32 and 33), one novel *B. taylorii* variant (Type 34) and one novel *B. birtlesii* variant (Type 30) (Table 3.4). One further variant (Type 31) did not closely match any known *Bartonella* species in GenBank; highest similarity was with BGA, but similarity was only moderate (90.6% pair wise sequence similarity). Details of novel pITS variants found in flea specimens here are given in the appendix (Table A4.4) and sequences have been submitted to GenBank (awaiting accession numbers).

Parasites detected in the commonly found flea, *C. n. vulgaris,* represented a range of wood mouse-exclusive, bank vole-exclusive and host-shared variants, and several parasites found in this flea species were not found in any others (Table 4.3). In contrast, *A. p. mustelae* and *M. turbidus* were not found to carry any wood mouse-exclusive variants but did harbour bank vole-exclusive and shared variants. The majority of variants found in both of these flea species were also detected in other flea species, but Types 8, 10 and 31 were exclusively found in *A. p. mustelae* and Type 28 in *M. turbidus* (Table 4.3). Only wood mouse-exclusive and host-shared variants were detected in *H. t. talpae*, all of which were detected in other flea species, and the single variant found in *R. pentacantha* was a wood mouse-exclusive variant, which was also found in two other flea species.

The Monte Carlo analysis found that for the majority of *Bartonella* variants, distribution across flea species did not differ significantly from random expectations, given the frequency with which flea species and *Bartonella* parasites were sampled; actual abundance of each variant in each flea species fell within the 95% confidence limits of simulated random distributions (Table 4.5). In line with this, the Mantel test found no significant correlation between *Bartonella* associations with host species and *Bartonella* associations with flea species (r = -0.005, p = 0.38), suggesting that interactions

between *Bartonella* variants and host species are not strongly structured by variation in host use by different flea species. However, the Monte Carlo analysis identified a single exception: the abundance of the bank vole-exclusive variant Type 08 (the sole variant of the species *B. rudakovii*) was greater than expected in *A. p. mustelae*, which was more often found to infect bank voles than wood mice, and lower than expected in *C. n. vulgaris*, which was more often found to infect wood mice (Table 4.5; Figure 4.5). This result is not explained by the collection of multiple *A. p. mustelae* specimens from the same Type 08-infected host, as multiple Type 08-positive *A. p. mustelae* were never collected from the same individual bank vole (see appendix, Tables A3 and A4).

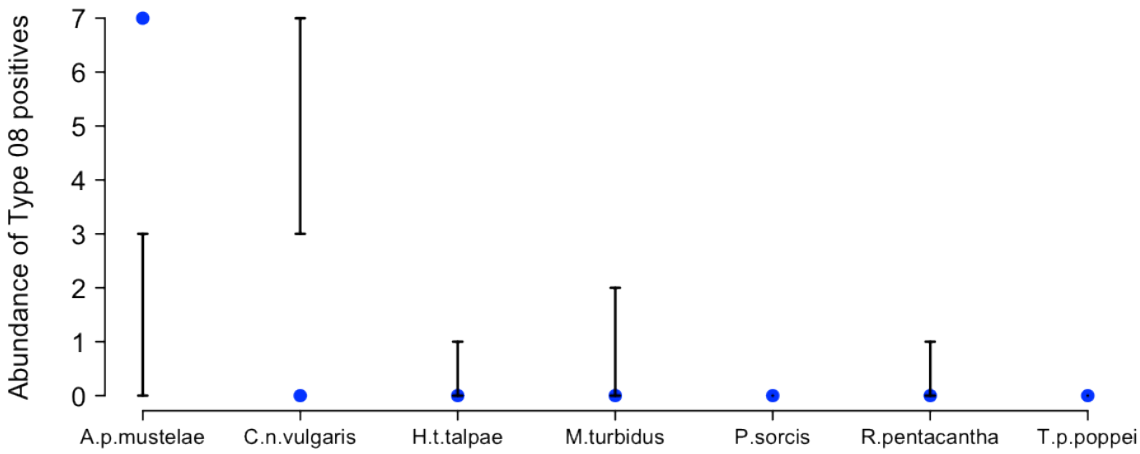

Figure 4.5: Results of a Monte Carlo simulation of the distribution of *Bartonella* variant Type 08 across flea species, assuming random associations based on their frequency within the sample. Bars are the 95% confidence limits based on the 100,000 runs of each simulation. Blue points are the observed abundances of variant Type 08 within each flea species. Observed abundance was greater than expected in *A. p. mustelae* and lower than expected in *C. n. vulgaris*.

Table 4.5: Results of Monte Carlo simulations of the distribution of *Bartonella* parasites across flea species, assuming random associations based on their frequency within the sample. C.L. shows the 95% confidence limits based on the 100,000 runs of each simulation. O.A. is the observed abundance of each parasite-flea species pairing. Highlighted cells indicate the parasite-flea species pairs in which the observed abundance falls outside of the 95% confidence limits of the simulation.

Bartonella variant	*A. p. mustelae*		*C. n. vulgaris*		*H. t. talpae*		*M. turbidus*		*P. sorcis*		*R. pentacantha*		*T. p. poppei*	
	C.L.	O.A.	C.L.	O.A.	C.L.	O.A.	C.L.	O.A.	C.L.	O.A.	C.L.	O.A.	C.L.	O.A.
Type 01	0 – 1	0	0 – 1	1	0 – 0	0	0 – 1	0	0 – 0	0	0 – 0	0	0 – 0	0
Type 02	0 – 3	3	2 – 6	2	0 – 1	0	0 – 1	1	0 – 0	0	0 – 1	0	0 – 0	0
Type 03	0 – 2	0	1 – 4	4	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 05	1 – 6	1	9 – 15	15	0 – 2	1	0 – 2	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 07	0 – 2	0	1 – 4	4	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 08	0 – 3	7	3 – 7	0	0 – 1	0	0 – 2	0	0 – 0	0	0 – 0	0	0 – 0	0
Type 09	0 – 2	0	1 – 3	2	0 – 1	0	0 – 1	1	0 – 0	0	0 – 1	0	0 – 0	0
Type 10	0 – 2	2	0 – 2	0	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 11	0 – 3	2	2 – 5	3	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 12	0 – 2	0	1 – 4	4	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 13	0 – 2	0	1 – 3	3	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 14	0 – 3	0	2 – 5	5	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 20	0 – 2	0	1 – 3	3	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 21	0 – 1	0	0 – 1	1	0 – 0	0	0 – 1	0	0 – 0	0	0 – 0	0	0 – 0	0
Type 23	0 – 3	0	2 – 5	3	0 – 1	1	0 – 1	0	0 – 0	0	0 – 1	1	0 – 0	0
Type 24	0 – 4	0	4 – 9	9	0 – 1	0	0 – 2	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 28	0 – 2	1	0 – 2	0	0 – 1	0	0 – 1	1	0 – 0	0	0 – 0	0	0 – 0	0
Type 30	0 – 3	0	2 – 5	5	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0
Type 31	0 – 1	1	0 – 1	0	0 – 0	0	0 – 1	0	0 – 0	0	0 – 0	0	0 – 0	0
Type 32	0 – 1	0	0 – 1	0	0 – 0	0	0 – 1	1	0 – 0	0	0 – 0	0	0 – 0	0
Type 33	0 – 1	0	0 – 1	1	0 – 0	0	0 – 1	0	0 – 0	0	0 – 0	0	0 – 0	0
Type 34	0 – 1	0	0 – 1	1	0 – 0	0	0 – 1	0	0 – 0	0	0 – 0	0	0 – 0	0

4.3.6 Assessing the potential for an ecological encounter barrier to between-species transmission

There were several examples of host-exclusive *Bartonella* variants being found within fleas that were collected from the alternative rodent species (Table 4.3). Wood mouse-exclusive *Bartonella* parasites (Types 12, 20, 24) were found in *C. n. vulgaris* collected from bank voles (Table 4.3, Figure 4.6b), and bank vole-exclusive *Bartonella* variants (Types 02, 01, 07) were found in *C. n. vulgaris* and *M. turbidus* (Type 02) collected from wood mice (Table 4.3, Figure 4.6b & 4.6d). Such patterns were not detected for *H. t. talpae* (Figure 4.6c) or *R. pentacantha* (Figure 4.6e); host-specific *Bartonella* parasites were only detected in fleas from the expected rodent species, although this may be due to a lack of power given the small number of parasites characterised in each case (one and two respectively). However, no such pattern was found in relation to *A. p. mustelae* either (Figure 4.6a), even though relatively more *Bartonella* parasites were characterised from specimens of this flea species (n=17) compared to *M. turbidus* (n=4) for which evidence of flea transfer was apparent. These patterns were consistent even when considering data from 2012 at MFG and RH only (Appendix Figure A4.1).

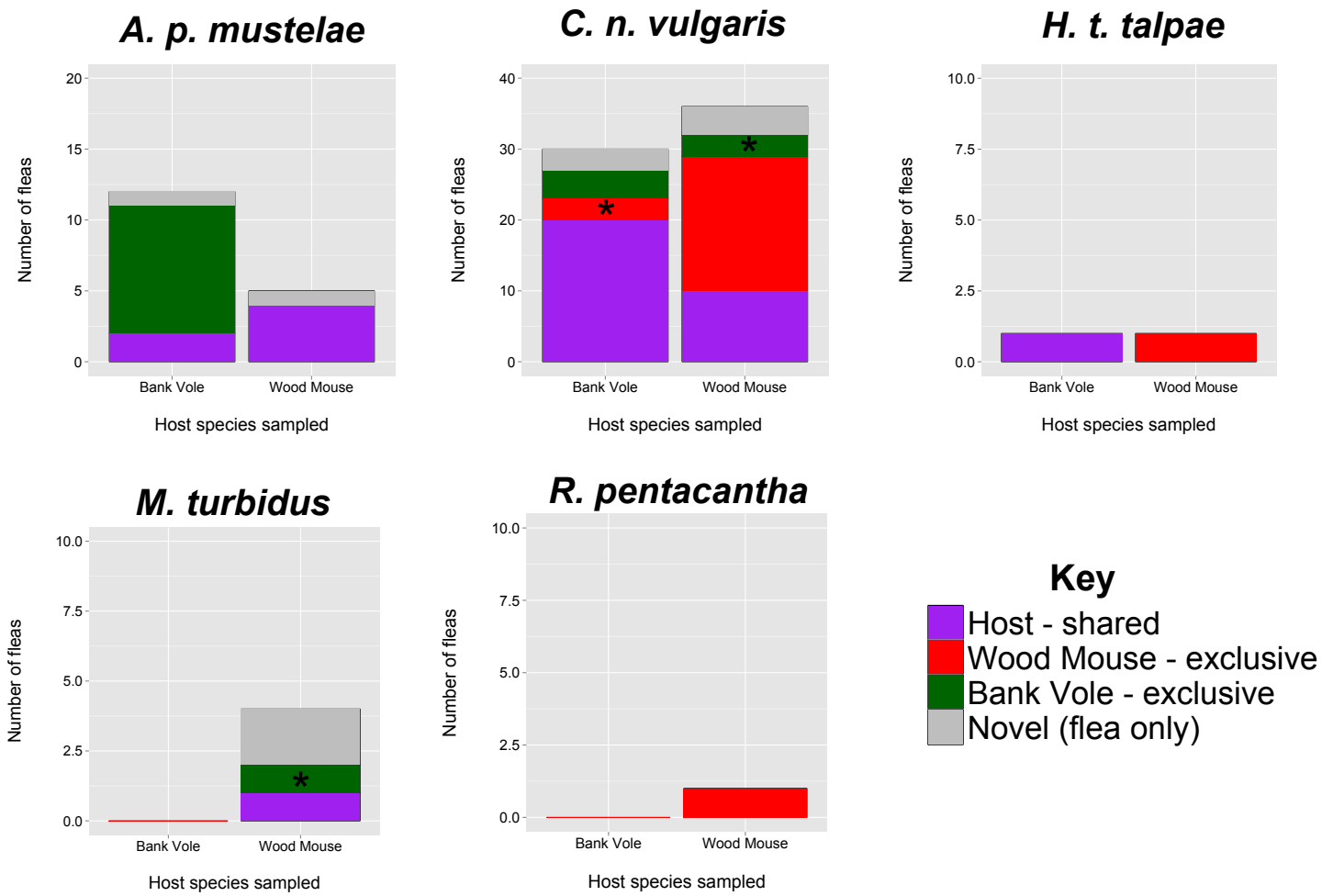

Figure 4.6: The number of *Bartonella*-positive fleas that were carrying variants of *Bartonella* previously found only in bank voles (green), wood mice (red), both rodent species (purple), or so far only in fleas (grey). Each flea species is shown separately.* indicates an occasion where host-exclusive variants were detected in fleas collected from the alternative host species. Wood mouse-specific variants were found in *C. n. vulgaris* fleas collected from bank voles, and bank vole-specific variants were found in *C. n. vulgaris* and *M. turbidus* fleas collected from wood mice.

4.4 Discussion

For the majority of *Bartonella* variants identified in the current study, including the 20 variants that appear to be host exclusive, there was no evidence that the associations of flea vectors with their rodent hosts would preclude their transmission between wood mice and bank voles. All except one very rare flea species (*T. p. poppei*) were collected at least once from both wood mice and bank voles, and while some *Bartonella* variants were detected exclusively within a single flea species, the distribution of these parasites in general did not depart from random given the heterogeneous nature of sampling. Furthermore, bank vole-exclusive *Bartonella* variants were detected in fleas collected from wood mice, and vice versa, which provides evidence that at least two flea species (*C. n. vulgaris* and *M. turbidus*) are able to transfer between individuals of different host species. Overall, these results suggest that fleas are not a barrier to between-species transmission of *Bartonella*, and so any host-exclusivity among *Bartonella* variants is more likely to arise through different compatibility across host species.

Several other studies have also found that the same flea species can often infect different rodent hosts within a community (Noyes *et al*., 2002; Telfer *et al*., 2005; Morick *et al*., 2010). It is perhaps unsurprising that similar assemblages of fleas infect these ecologically similar rodent species (wood mice and bank voles). The survival and development of both larval and adult fleas are sensitive to environmental conditions (e.g. Zwolak *et al*., 2013) and affected by air temperature, relative humidity and substrate structure (Marshall, 1981). The distribution of flea species may therefore reflect the suitability of abiotic conditions within certain habitats, rather than available host species per se. However, the reproductive success of flea species in Israel has been shown to depend on the species identity of the host on which it feeds (Krasnov *et al*., 2004a; Khokhlova *et al*., 2012). Such heterogeneous fitness consequences may explain why, in the present study, some flea species were more often collected from either wood mice (*C. n. vulgaris*) or bank voles (*A. p. mustelae* and *R. pentacantha*). Certain host species may be more adapted to resisting the attack of certain flea species, due to immunological defences (Wikel, 1999) or grooming behaviour (Hawlena *et al*., 2007). Furthermore, some flea species may display adaptive specialisation enabling them to better circumvent the physical defences of specific host species. For example, some

fleas possess spines and setae that aid attachment to hosts' hairs and help them to resist host grooming, (Amin, 1982). The number and arrangement of such setae may determine the ability of the different flea species identified here to more successfully attach to the fur of wood mice or bank voles. Indeed, Tompkins & Clayton (1999) identified similar fine-scale morphological differences between species of parasitic mite, which determined their degree of adaptation to different species of swiftlet.

As well as finding little evidence of strict species-specific relationships between hosts and fleas, I also found that the distributions of the vast majority of *Bartonella* variants were random in relation to flea species. Strict relationships between parasites and vectors have been refuted in other disease systems; Kimura *et al*. (2010) found that several of the same lineages of avian *Plasmodium* parasites were found in multiple sympatric mosquito species, and Noyes *et al*., (2002) found that multiple species of flea carried the same rodent *Trypanasoma* parasites in the wild. In the present study, several different *Bartonella* variants were also detected in the same flea species, which is consistent with previous work in other systems (Abbot *et al*., 2007; Brinkerhoff *et al*., 2010; Morick *et al*., 2010). Such patterns suggest that fleas do not restrict the transmission of *Bartonella* parasites between wood mice and bank voles, as even though certain species of flea were associated more with one host species than the other, it is unlikely that they are the only species able to carry particular *Bartonella* variants. An absence of specific relationships between flea species and *Bartonella* parasites is perhaps unsurprising, as current knowledge suggests that *Bartonella* spp. are essentially commensal organisms within the flea, replicating within the flea gut (Chomel *et al*., 2009) and causing little or no detriment to the vector (Morick *et al*., 2013). With potentially few cellular interactions between the parasite and flea, it seems unlikely that specific relationships would develop.

However, there was one clear exception to the pattern of random associations between *Bartonella* variants and flea species. Variant Type 08, the single genetic variant representing the vole-excusive species *B. rudakovii* (see Chapter 3), was positively associated with *A. p. mustelae* and negatively associated with *C. n. vulgaris*. In fact, this variant was only detected in *A. p. mustelae*, strongly suggesting that transmission of *B. rudakovii* between individual rodents, and indeed between rodent species, solely depends on this flea species. This variant was bank vole-exclusive (Chapter 3), and *A. p.*

mustelae fleas displayed a preference for bank voles. Together, this suggests a potential flea-mediated barrier to the transmission of *B. rudakovii* between bank voles and wood mice.

Why *B. rudakovii* should be associated with a single flea species, while other *Bartonella* species and genetic variants were not, is unclear. Certainly, further sampling of fleas within these rodent communities, and characterisation of the *Bartonella* parasites they carry, may uncover other strict flea-*Bartonella* associations. However, a genuine association may arise if the developmental cycle of *B. rudakovii* differs from other *Bartonella* species identified here, such that carriage within the vector is accompanied by a greater degree of interaction between parasite and flea (e.g. as in *Plasmodium*-mosquito interactions, Marois, 2011). Given the high level of dissimilarity between the pITS region of *B. rudakovii* and other *Bartonella* variants that I have characterised (Chapter 3), it is possible that *B. rudakovii* also differs at regions of the *Bartonella* genome involved with vector interactions. Differences between closely-related microparastes in their complex within-vector dynamics has been demonstrated for other parasite species (e.g. Alavi *et al*., 2003). *B. rudakovii* may have become adapted to *A. p. mustelae* and not other flea species if contact with only this flea species in the past was more frequent. Further investigations of the within-flea stage of the *Bartonella* life cycle would shed light on whether the level of flea interaction varies between *Bartonella* species and variants, and whether this could explain the specific association between *B. rudakovii* and *A. p. mustelae* identified here.

With the exception of *B. rudakovii*, evidence to suggest specific associations between *Bartonella* species/variants, flea species and host species was lacking. In addition, four fleas collected from wood mice harboured bank vole-exclusive *Bartonella* variants, and three fleas collected from bank voles tested positive for wood mouse-exclusive *Bartonella* variants, providing preliminary evidence that at least two flea species (*C. n. vulgaris* and *M. turbidus*) are able to transfer between individuals of different host species. This refutes a flea-mediated barrier to between-species *Bartonella* spp. transmission based on a lack of ecological opportunity for flea transfer, despite differences between wood mice and bank voles in terms of microhabitat preference (Bergstedt, 1965; Geuse, 1985), range size and dispersal patterns (Kikkawa, 1964; Crawley, 1969; Carslake *et al*., 2005), period of daily activity (Greenwood, 1978) and

diet preferences (Watts, 1968; Canova, 1993). Noyes *et al*. (2002) also found evidence of interspecific flea transfer in a similar study system, as a flea collected from a wood mouse at a site in northwest England was carrying a bank vole-specific *Trypanasoma* parasite. Similarly, Telfer *et al*. (2005) found that fleas collected from bank voles in Ireland were carrying *Bartonella* parasites despite infections only being detected in sympatric wood mice and never in bank voles. Experimental mark-release-recapture studies of fleas, where individual fleas were radioactively labelled or marked by tarsal clipping, have also provided more direct evidence of flea transfer between multiple rodent species (Hartwell *et al*., 1957; Rödl, 1979).

At present, the possible mechanisms of flea transfer between wood mice and bank voles are unknown. One possibility is that transfer may occur through direct host contact. Experimental enclosure studies found that the proportion of fleas transferred between two rodent host species (house mouse, *Mus musculus*, and the midday jird, *Meriones meridianus*) was positively correlated with the number and cumulative duration of tactile contacts between them (Krasnov & Khokhlova, 2001). If this finding can be extrapolated to other rodent species within their natural habitat, then results here suggest that physical contacts between wood mice and bank voles at my study sites may occur more frequently than anticipated. However, the most common flea species identified here (*C. n. vulgaris*) has been classified as a "nest flea" (spending a large proportion of its life within the host's nest, and only remaining on-host for the duration of feeding; Marshall, 1981; Krasnov, 2008). Thus a more likely mechanism of flea transfer between wood mice and bank voles (and indeed between individuals of the same species), may be the successive use of the same burrow system by different host species. Wood mice and bank voles have been known to visit, and even usurp, each other's burrows (Flowerdew, 1993), and transfer of radioactively labelled fleas via host-nest contact has been demonstrated in a semi-natural experimental study (Rödl, 1979). Interestingly, I found evidence of flea transfer in both directions here (bank vole to wood mouse, and wood mouse to bank vole), but it is possible that transfer occurs more often in one direction than the other, if one species is more likely to visit the burrows of the other (e.g., if wood mice tend to usurp burrows of bank voles rather than vice versa), causing a predominantly uni-directional flow of fleas between the species. If true, this would have important implications for the direction of *Bartonella* transmission within the community.

In light of the lack of support for flea-mediated barriers to *Bartonella* transmission, it seems that wood mice and bank voles are challenged by many of the same *Bartonella* parasites, including multiple variants of the same *Bartonella* species. In spite of this, each host species is only infected by a distinct subset of these parasites (Chapter 3), suggesting that each is susceptible to infection by only some *Bartonella* species/variants. A previous study of *Bartonella* infections in Irish rodent communities found that bank voles were not infected with any *Bartonella* parasites despite infections being found in sympatric wood mice, infected by the same flea species, and the fact that bank voles in the UK were apparently susceptible to the same *Bartonella* species in question (Telfer *et al*., 2005). As bank voles are a relatively recent introduction to Ireland, among other reasons the authors proposed that these rodents might not be susceptible to these geographically distinct Irish strains. The evidence presented here and in Chapter 3 suggests that bank voles in the UK may also be unsusceptible to many of the strains of *Bartonella* infecting wood mice with which they share an established sympatric existence. Host-specific *Bartonella* compatibility has also been demonstrated experimentally in a different rodent system: when inoculated with different strains of *Bartonella*, white-footed mice (*Peromyscus leucopus*) and cotton rats (*Sigmodon hispidus*) in the USA only developed bacteremia when infected with strains originally taken from the same or phylogenetically closely related rodent species (Kosoy *et al*., 2000). Similar reciprocal infection experiments would be useful to confirm the putative physiological incompatibilities between *Bartonella* variants and each rodent species here. Furthermore, while current associations between bank voles, *A. p. mustelae* and *B. rudakovii* may preclude transmission of *B. rudakovii* between bank voles and wood mice, the latter may still be susceptible to *B. rudakovii*, and this should be investigated. If they are susceptible, changes to these flea-mediated associations could result in the emergence of *B. rudakovii* into wood mouse populations in the future (i.e. a "potential multihost" pathogen according to Fenton & Pedersen, 2005). However, *A. p. mustelae* was occasionally found to infect wood mice, so there is already some opportunity for wood mouse exposure to *B. rudakovii* in theory.

Assuming that *Bartonella* species and variants vary in their compatibility with wood mice and bank voles, the mechanisms underlying this physiological host-specificity warrant further investigation. Host specificity of different *Bartonella* species across

distantly related mammalian hosts has been shown to be associated with the ability of bacteria to adhere to the erythrocytes of their hosts; a process mediated by a cluster of genes within the Trw-type IV secretion system (Saenz *et al*., 2007; Vassier-Taussat *et al*., 2010). The *Bartonella* variants in the present study were characterised according to their sequence at a partial fragment of the non-coding ITS region (referred to as the "pITS" region in Chapter 3), and so these sequences do not directly influence host compatibility. However, the variation identified at the ITS region may reflect variation at functionally important regions of the genome (e.g., the Trw-type IV secretion loci, or perhaps at others related to the evasion of host-specific immunity; Saenz *et al*., 2007). Such variation in parasite-host compatibility has been demonstrated between different closely related genotypes of several parasites (e.g. Lopez *et al*., 2003; Fickenscher *et al*., 1997).

Compatibility between some *Bartonella* variants and host species and not others may indicate a long history of co-evolution between compatible pairs. Signals of co-evolution between *Bartonella* parasites and rodents have indeed been demonstrated previously in other rodent systems (Lei *et al*., 2014). Alternatively, a lack of *Bartonella* transmission between wood mice and bank voles may have led to the divergence of previously generalist *Bartonella* lineages, such that the parasites have become locally adapted to infect the host species with which they have most frequent contact (Poulin *et al*., 2008). Restricted transmission of parasites within isolated groups of individuals reduces gene flow and may generate genetic structure within a parasite population (Bruyndonckx *et al*., 2009; Ruiz-Gonzalez *et al*., 2012). As parasites are often more numerous and have shorter generation times than their hosts (Hamilton *et al*., 1990), genetic bottlenecks may give rise to local adaptation of parasites, where a parasite is only able to exploit a single host species even if the opportunity to exploit other hosts arises (Yourth & Schmid-Hempel, 2006; Little *et al*., 2006). Although I find no evidence of current flea-mediated encounter barriers, such blocks in transmission may have been previously present and resulted in isolated within-host *Bartonella* transmission. Furthermore, while incompatibility may have arisen between some *Bartonella* parasites and host species, the random nature of genetic bottlenecks means that some variants may still be infectious to both wood mice and bank voles, which is what was observed here. If such mechanisms do underpin the patterns of *Bartonella*-host compatibility here, it suggests that the very nature of this compatibility may be

transient, and subject to the dynamic interactions of hosts and fleas within the community, which may change over time (Ruiz-Gonzalez *et al*., 2012). If so, then a similar survey of host-*Bartonella* associations carried out at a different point in time may reveal a different subset of host-exclusive and host-shared variants, or even that the same variants had changed their patterns of host exclusivity/generalism.

Several novel *Bartonella* variants were detected in fleas that had so far not been identified from either wood mice or bank voles. Several studies have found that fleas often carry a broader range of *Bartonella* parasites than are found in the hosts they infect (Abbot *et al*., 2007; Brinkerhoff *et al*., 2010). It is possible that the novel variants identified here circulate within wood mouse and/or bank vole populations, but at a very low prevalence. Genetic characterisation of *Bartonella* infections within these host species at the same study sites was extensive (Chapter 3), but some variants were represented by only a single infection, suggesting that rare variants may indeed circulate. Alternatively, these flea-only variants may be associated with host species other than wood mice or bank voles. *Bartonella* spp. are known to infect other small woodland mammals, including field voles, *Microtus agrestis* (Telfer *et al*., 2007b) and common shrews, *Sorex araneus* (Bray *et al*., 2007), and both of these potential host species have been occasionally trapped at the field sites used in this study.

The DNA of multiple *Bartonella* species was detected in 13 fleas (12% of *Bartonella*-positive fleas). A study of *Polygenis gwyni* fleas parasitizing cotton rats (*Sigmodon hispidus*) in the USA also found that the presence of multiple *Bartonella* genotypes in individual fleas was common (Abbot *et al*., 2007). A flea may pick up multiple *Bartonella* infections if it feeds on an individual that is coinfected, which is common within wood mice in this study system (Devevey *et al*., in prep). Alternatively, a flea may feed sequentially on multiple individuals that are infected with different *Bartonella* parasites. In addition, some evidence suggests that *Bartonella* parasites may be transmitted vertically from adults to offspring, either transovarially (Brinkerhoff *et al*., 2010), or when flea larvae consume the infected faeces of adult fleas (Morick *et al*., 2013). Regardless of how flea coinfection arises, the presence of multiple *Bartonella* genotypes within individual vectors may promote genetic diversification of *Bartonella* parasites via recombination (Paziewska *et al*., 2011; Berglund *et al*., 2010), which may

explain why multiple pITS variants of the same *Bartonella* species were found to infect the same host species.

Prevalence of *Bartonella* was significantly lower in *R. pentacantha* compared to other flea species that were equally sampled, suggesting that the vector competency of this species is comparatively low. However, the seasonal dynamics of *R. pentacantha* differs from other flea species found here, as it is more abundant during late autumn and winter compared to the high summer abundance of other flea species (Harris *et al*., 2009; Whitaker, 2007). As many rodent fleas are nest-dwellers and rely on detecting host odour and CO_2 to locate their hosts at close proximity (Marshall, 1981), transfer between individuals, and therefore contact with hosts harbouring *Bartonella* infections, depends largely on the movement patterns of host individuals. Lower prevalence in *R. pentacantha* may therefore reflect the lower rates of activity among rodent hosts during the period when this flea is most abundant (Wolton, 1983), which may reduce the contact rate between fleas and infectious hosts, and therefore result in an overall lower rate of transmission within the system.

The associations between flea and host species identified here are based on the presence of fleas on the host body. However, the presence of a flea on a rodent may not necessarily indicate its suitability as a blood meal source. Using blood meal analysis would allow more fine-scale assessments of host use by different flea species, as has been done for flea vectors of plague (Woods *et al*., 2009; Franklin *et al*., 2010) and dipteran vectors of various microparasites (Hassan *et al*., 2003; Kilpatrick *et al*., 2006; Hamer *et al*., 2009). Furthermore, while the abundance of fleas collected from rodents has been shown to correlate positively with flea abundance in the environment of that host (Krasnov *et al*., 2004c), accurate estimates of infestation per host may be compromised by the fact that fleas can leave the bodies of captured animals due to host stress (Marshall, 1981). Host-preferences of different flea species, assessed via relative abundance of fleas associated with each host species, may therefore profit from direct surveys of fleas present within host nests. It is also possible that detection of *Bartonella* DNA within a flea does not necessarily prove vector competency, as physiological alterations to *Bartonella* bacteria within the flea gut may render the parasite non-functional. The PCR method used here to detect *Bartonella* infections does not provide information on the bacterial load within the fleas, which may vary between flea species

or even be affected by blood meal source (Eisen *et al*., 2008), thus affecting vector competence. Finally, DNA yield from a few individual fleas was very low, and it is possible that detection of *Bartonella* DNA was compromised in these cases. However, low DNA yield did not occur more often for particular species of flea, therefore even if some false-negatives did occur, it would not have affected the overall associations between flea species and *Bartonella* parasites that were identified here.

This study has provided important insights into associations between fleas, *Bartonella* parasites and rodent hosts, increasing our understanding of *Bartonella* transmission within this natural rodent community. Broad patterns of flea sharing between wood mice and bank voles, coupled with largely random associations between flea species and *Bartonella* variants, indicates that independent circulation of *Bartonella* parasites within populations of these two sympatric rodent species, for the most part, is not the result of current flea-mediated barriers to between-species transmission, and possibly implicates a role for a parasite-host compatibility filter in limiting host range for a number of variants in this system. These findings are based on observational data, and future work should consider the use of experimental manipulations to corroborate these patterns, and investigate the potential for host-related effects on flea dynamics to impact risk of *Bartonella* infection in the community.

4.5 References

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4.6 Appendix

Table A4.1: Species identity and infection status of multiple fleas taken from the same individual bank vole. Table continued on next page.

Host ID	Session	Flea species	No. of flea species	Flea *Bartonella* status	All same infection status?
67011289	4	H. t. talpae	1	None	Yes
		H. t. talpae		None	
67069563	4	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
79617560	4	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
		C. n. vulgaris		None	
79631074	4	C. n. vulgaris	1	Type 14	Yes
		C. n. vulgaris		Type 14	
		C. n. vulgaris		Type 14	
79635855	1	A. p. mustelae	1	None	Yes
		A. p. mustelae		None	
79769542	3	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
		C. n. vulgaris		None	
79769790	2	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
79787832	6	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
66875864	4	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
67075352	4	C. n. vulgaris	1	None	No
		C. n. vulgaris		Type 02	
67285795	5	C. n. vulgaris	1	None	No
		C. n. vulgaris		Type 05	
		C. n. vulgaris		*B. taylorii*	
66820622	6	C. n. vulgaris	1	Type 05	No
		C. n. vulgaris		Type 05	
		C. n. vulgaris		Type 30	
79795281	4	C. n. vulgaris	1	None	No
		C. n. vulgaris		*B. taylorii* + *B. birtlesii*	
66880631	8	C. n. vulgaris	2	None	Yes
		R. pentacantha		None	
		R. pentacantha		None	
		R. pentacantha		None	
67070366	6	A. p. mustelae	2	None	Yes
		C. n. vulgaris		None	
79777610	1	A. p. mustelae	2	None	Yes
		C. n. vulgaris		None	
67277259	2	C. n. vulgaris	2	None	No
		A. p. mustelae		Type 08	

Table A4.1: Continued from previous page.

Host ID	Session	Flea species	No. of flea species	Flea *Bartonella* status	All same infection status?
79614105	6	A. p. mustelae	2	None	No
		A. p. mustelae		None	
		C. n. vulgaris		Type 03	
79619778	4	A. p. mustelae	2	None	No
		C. n. vulgaris		Type 14	
79785879	4	C. n. vulgaris	2	None	No
		C. n. vulgaris		None	
		C. n. vulgaris		None	
		C. n. vulgaris		None	
		C. n. vulgaris		Type 11	
		C. n. vulgaris		*B. taylorii* + *B. birtlesii* + E	
		M. turbidus		None	
Untagged	4	C. n. vulgaris	2	None	No
		C. n. vulgaris		Type 30	
		C. n. vulgaris		*B. grahamii* + E	
		A. p. mustelae		*B. taylorii* + E	
79629354	3	A. p. mustelae	3	None	Yes
		C. n. vulgaris		None	
		C. n. vulgaris		None	
		C. n. vulgaris		None	
		R. pentacantha		None	
79611354	6	A. p. mustelae	3	Type 02	No
		C. n. vulgaris		Type 03	
		H. t. talpae		*B. grahamii*	

Table A4.2: Species identity and infection status of multiple fleas taken from the same individual wood mouse.

Host ID	Session	Flea species	No. of flea species	Flea *Bartonella* status	All same infection status?
66875864	4	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
67076621	6	C. n. vulgaris	1	None	Yes
		C. n. vulgaris		None	
79620777	4	C. n. vulgaris	1	Type 23	Yes
		C. n. vulgaris		Type 23	
67123625	4	C. n. vulgaris	1	None	No
		C. n. vulgaris		Type 05	
		C. n. vulgaris		Type 20	
79609832	6	C. n. vulgaris	1	Type 13	No
		C. n. vulgaris		Type 24	
79795888	7	C. n. vulgaris	1	None	No
		C. n. vulgaris		*B. taylorii* + *B. birtlesii*	
79635087	1	C. n. vulgaris	1	None	No
		C. n. vulgaris		Type 05	
		C. n. vulgaris		*B. grahamii* + *B. taylorii*	
79638019	5	C. n. vulgaris	1	Type 11	Unknown
		C. n. vulgaris		*B. taylorii*	
67089554	6	C. n. vulgaris	2	None	Yes
		T. p. poppei		None	
Untagged	4	C. n. vulgaris	2	None	No
		C. n. vulgaris		Type 30	
		C. n. vulgaris		*B. grahamii* + E	
		A. p. mustelae		*B. taylorii* + E	
52806809	5	H. t. talpae	2	None	No
		C. n. vulgaris		Type 12	
		C. n. vulgaris		Type 12	
66864345	5	C. n. vulgaris	2	None	No
		C. n. vulgaris		None	
	6	C. n. vulgaris		None	
		C. n. vulgaris		*B. taylorii*	
	7	C. n. vulgaris		None	
		R. pentacantha		None	
66881347	4	C. n. vulgaris	2	None	No
		C. n. vulgaris		None	
		C. n. vulgaris		Type 24	
		M. turbidus		Type 28	
79786569	6	T. p. poppei	2	None	No
		C. n. vulgaris		Type 24	

Table A4.3: The number of fleas that were found to be carrying multiple putative species of *Bartonella* parasites. Ticks in the relevant columns represent the combination of parasites found in each case.

	***B. rudakovii* / BGA**	***B. birtlesii***	***B. taylorii***	***B. grahamii***
***B. doshiae* / *B. doshiae*-like**	0	0	0	0
B. grahamii	5	1*	2	
B. taylorii	1	3		
B. birtlesii	2**			

* *B. grahamii* and *B. birtlesii* only seen in coinfection along with *B. rudakovii*/BGA (n=1)
** *B. rudakovii* and *B. birtlesii* only seen in coinfection along with either *B. grahamii* (n=1) or *B. taylorii* (n=1)

Table A4.4: The six novel *Bartonella* partial 16S-23S ITS sequence types detected in this study. Sequence types are grouped into *Bartonella* species groups based on their closest match to known *Bartonella* species within GenBank. The accession numbers, source and description of these closest matches are given, along with percentage identity and coverage.

***Bartonella* species**	**pITS variant**	**# base pairs**	**pITS size category**	**Accession # of closest BLAST result**	**% Identity**	**% Coverage**
B. grahamii	Type 28	282	B	AJ269790.1	100	100
	Type 32	282	B	AJ269790.1	99	100
	Type 33	282	B	JN810855.1	99	100
B. taylorii	Type 34	316	C	AJ269796.1	99	100
B. birtlesii†	Type 30	338	D	KC907381.1	99	100
Unknown	Type 31	426	E	HM596449.1	91	100

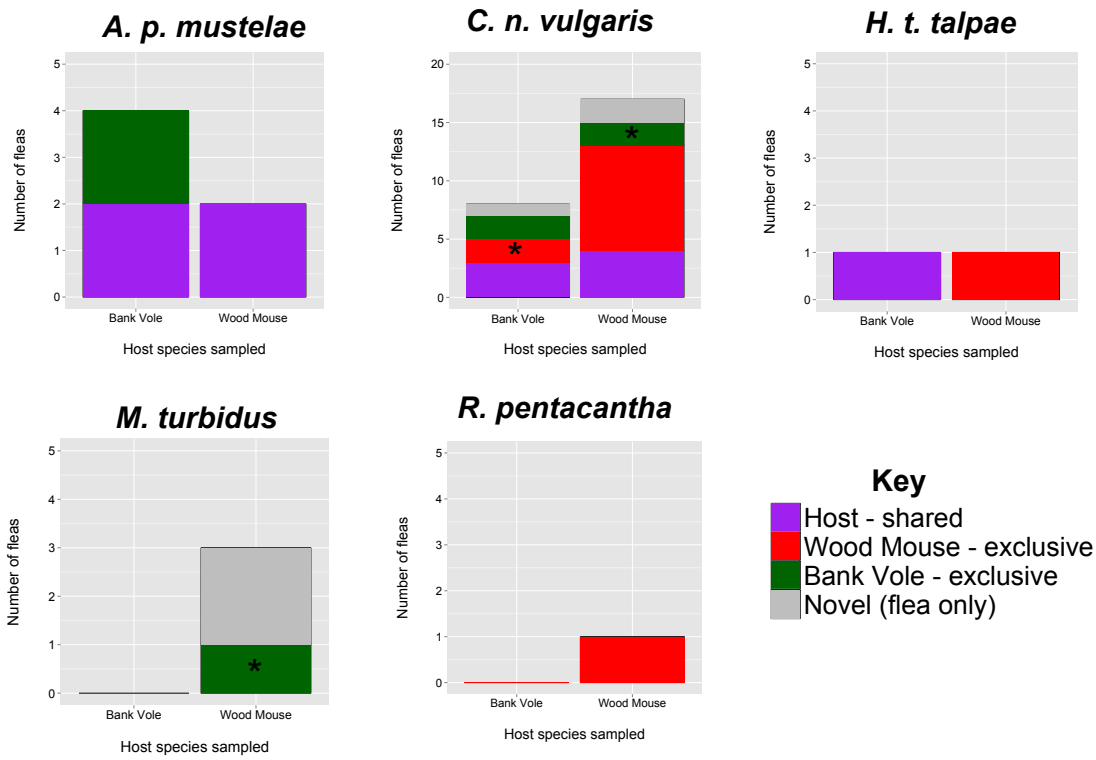

Figure A4.1: The number of *Bartonella*-positive fleas that were carrying variants of *Bartonella* previously found only in bank voles (green), wood mice (red), both rodent species (purple), or so far only in fleas (grey): Each flea species is shown separately. * indicates an occasion where host-exclusive variants were detected in fleas collected from the alternative host species. Wood mouse-specific variants were found in *C. n. vulgaris* fleas collected from bank voles, and bank vole-specific variants were found in *C. n. vulgaris* and *M. turbidus* fleas collected from wood mice. **Data are from 2012 only, and from MFG and RH only.**

Chapter 5
Experimental manipulation of between-species *Bartonella* transmission within natural rodent communities.

5.1 Introduction

Most parasites appear to infect multiple host species in nature (Cleaveland *et al*., 2001; Woolhouse *et al*. 2001; Pedersen *et al*., 2005; Begon *et al*., 1999). Transmission between host species may be necessary for a parasite to persist within certain host species populations, and the rate at which this occurs may therefore determine an individual's risk of infection (Holt *et al*., 2003; Dobson, 2004). However, host species within a community are unlikely to contribute equally to parasite transmission, due to differences in abundance or underlying heterogeneities related to exposure, susceptibility and immunity (Haydon *et al*., 2002; Altizer *et al*., 2003; Kilpatrick *et al*., 2006; Streicker *et al*., 2013). Therefore certain species may contribute disproportionately to transmission and be nearly completely responsible for the persistence of a parasite within a community and the infection rates of other sympatric host species (Streicker *et al*., 2013). Identifying such "key hosts" therefore offers a means to appropriately target control interventions to maximise success (e.g. Rudge *et al*., 2013; Donnelly et al. 2006; Kaare et al. 2009).

Identifying the extent to which sympatric host species transmit generalist parasites to each other, and therefore the structure of the parasite "maintenance community" (Haydon *et al*., 2002), is challenging however, as similar patterns of parasite prevalence within host populations may result from very different underlying transmission processes (Fenton & Pedersen, 2005; Viana *et al*., 2014). At one extreme, a parasite may only be maintained within a host species population due to regular spillover transmission from another key host species (i.e. an "apparent" multi-host generalist; Fenton & Pedersen, 2005). At the other extreme, transmission may occur equally within and between host species, with each species contributing to parasite maintenance within the community as a whole (i.e. a "true" multi-host generalist; Fenton & Pedersen,

2005). As a result of the underlying complexity of parasite maintenance communities, identifying key transmission hosts, and therefore the most effective targets for parasite control interventions, is likely to require a combination of approaches beyond observational studies of disease incidence (Viana *et al*, 2014).

One potentially useful approach for identifying key transmission hosts is to manipulate transmission between host species within the community and to observe the effect on disease prevalence in the host species of interest (i.e. the "target" host according to the terminology of Haydon *et al*., 2002). To such ends, applied disease control interventions (e.g., physical separation of host species, or chemotherapeutic or vaccination programmes that reduce parasite prevalence in one host species), offer useful insights (Viana *et al*., 2014). For example, fenced hunting estates in Spain have prevented contact between fenced-in wild ungulates (e.g. red deer and wild boar) and fenced-out domestic livestock for up to twenty years, but *M. bovis* infections were found within both wild and domestic animals, indicating that each group can maintain infections in the absence of transmission from the other (Gortazar *et al*., 2005). In another example, mass vaccination of cattle against Rinderpest Virus eventually saw elimination of this disease from cattle and sympatric wildlife in Africa (Roeder *et al*., 2013), indicating that cattle, and not wildlife, were the key transmission hosts.

While applied control interventions offer useful insights into the nature of parasite transmission within natural multi-host communities, such quasi-experiments are undeniably limited in the broader understanding they can provide, as interventions that increase knowledge of the disease system to optimise future control may not meet the primary goals of control interventions, i.e. to reduce the prevalence of disease in the target host species as rapidly as possible (Allen & Stankey, 2009). Model host-parasite systems that are amenable to the manipulation of transmission are therefore useful for studying host-parasite dynamics without the complications that accompany applied control interventions.

Bartonella infections within woodland rodent communities offer an ideal opportunity to study the transmission dynamics of endemic parasites using transmission manipulation experiments within a natural multi-host community. The Bartonellae are bacterial haemoparasites of a diverse range of mammalian host species (Breitschwerdt &

Kordick, 2000). Several different species have been detected within wild rodents (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al*., 2007b; Knap *et al*., 2007; Bray *et al*., 2007; Gil *et al*., 2010; Welc-Faleçiak *et al.,* 2010; Paziewska *et al*, 2012), and the primary means of transmission is believed to be via the feeding activity of fleas (Bown *et al*., 2004; Morick *et al*., 2011). Wood mice and bank voles are common woodland rodents in the UK and often occur in sympatry. Their small size and fidelity to relatively restricted spatial areas (as determined by the often spatially patchy distribution of suitable woodland habitat; Kikkawa, 1964), means that individuals, the populations they constitute, and their parasitic infections can be well characterised.

In the UK, several species of *Bartonella* have been found to circulate endemically within sympatric populations of wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*), and their general ecology has been well characterised in several studies (Birtles *et al*., 2001; Telfer *et al*., 2005; Telfer *et al.,* 2007a; Telfer *et al*., 2010; Chapter 2). Importantly, several *Bartonella* species (*B. grahamii*, *B. taylorii* and *B. birtlesii*) are reported to infect both host species at the same site (Birtles *et al*., 2001; Telfer *et al.,* 2007a; Chapter 2) and are transmitted between individuals via host-generalist fleas (Chapter 4). However, the degree to which transmission of *Bartonella* spp. occurs between wood mice and bank voles, and whether either host species is a key transmission host for any of these parasites, remains unresolved. Previous longitudinal studies have found some evidence for the density of one host species affecting risk of infection in the other host species, therefore suggesting possible between-host species transmission routes, but this evidence was inconclusive in many cases (Telfer *et al*., 2007a; Chapter 2). Furthermore, recent work established that generalist *Bartonella* species at several sites in northwest England comprised a complex of genetic variants (defined by the 16S-23S rRNA pITS region; Chapter 3), and that variants of the same *Bartonella* species had different distributions among host species. Some variants were found only in wood mice (wood mouse-exclusive), some only in bank voles (bank vole-exclusive), and only a minority were found to infect both (host-shared; Chapter 3). This raises the possibility that transmission of apparently generalist species of *Bartonella* between wood mice and bank voles may be limited, and therefore not necessary for the persistence of infection within either host species.

To assess whether transmission of *Bartonella* occurs between wood mice and bank voles, I attempted to experimentally reduce the rate at which the various *Bartonella* species were transmitted between these host species in their natural environment. To achieve this, I used targeted treatment to remove flea vectors from bank voles, in an attempt to reduce the bank vole-generated force of infection (i.e. infectious fleas arising from bank voles) within this system (see Figure 5.1 for details). By observing how this manipulation affected infection risk in bank voles and wood mice, I hoped to identify whether transmission from bank voles to wood mice is common for different *Bartonella* species and variants, and whether bank voles are a key transmission host for any of these parasites. Specifically, I hypothesised that transmission of *Bartonella* species between bank voles and wood mice is limited to the few host-shared sub-specific *Bartonella* pITS variants identified in Chapter 3. As the flea vectors appear to be host-generalists (Chapter 4), it was hypothesised that wood mice and bank voles contribute equally to the size of the vector community; therefore treatment of the bank vole population was not expected to reduce infection risk in either host species simply by reducing the size of the vector population feeding on the wood mice. Rather, a reduction in risk would result from a reduction in the prevalence of infection in the flea community. I therefore predicted the following as a result of targeted anti-flea treatment of bank voles:

1. Risk of either host species being infected with fleas will be unaffected.
2. Risk of bank vole infection with each *Bartonella* species will be reduced overall, but host-shared variants may persist as a result of continued transmission from wood mice (Figure 5.2a).
3. Risk of wood mouse infection with each *Bartonella* species will not be reduced overall, but the risk of infection with host-shared variants may be reduced if transmission from bank voles is necessary for persistence (Figure 5.2b).

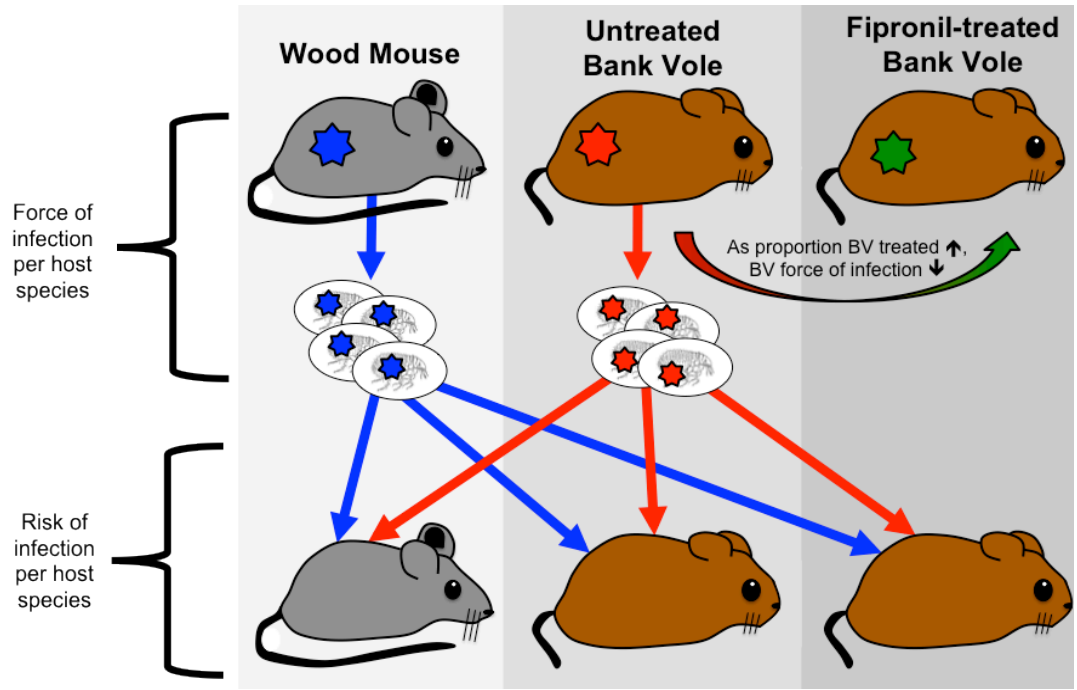

Figure 5.1: The hypothesised effect of Fipronil treatment on *Bartonella* transmission between wood mice and bank voles. Stars represent *Bartonella* infection, and are colour-coded according to the type of host being infected. Fleas become infected with the appropriate *Bartonella* according to the type of host on which they feed. Force of infection (number of infectious fleas) produced by wood mice is unaffected by treatment of bank voles. Infectious fleas that arise from biting infected wood mice are able to infect other wood mice, and bank voles, regardless of whether the bank vole has been treated (blue arrows). Some bank voles remain untreated, and their contribution to the bank vole force of infection is unchanged. Infectious fleas that arise from biting an untreated bank vole are able to infect wood mice and other bank voles, regardless of whether they have been treated (red arrows). Fipronil-treated bank voles do no contribute to the bank vole force of infection, because fleas that bite them die shortly afterwards, and are removed from the pool of infectious fleas. Assuming that flea death occurs before a flea is able to bite another host, fleas that become infectious as a result of biting a Fipronil-treated bank vole will not be able to transmit infection to any individuals. As the proportion of the bank vole population that are treated increases, fewer bank voles remain untreated, therefore fewer are able to contribute to the bank vole force of infection. The rate of transmission from the bank vole population (red arrows) will therefore decline. Picture credits: A.B. Pedersen (wood mouse, bank vole), all-free-download.com (flea).

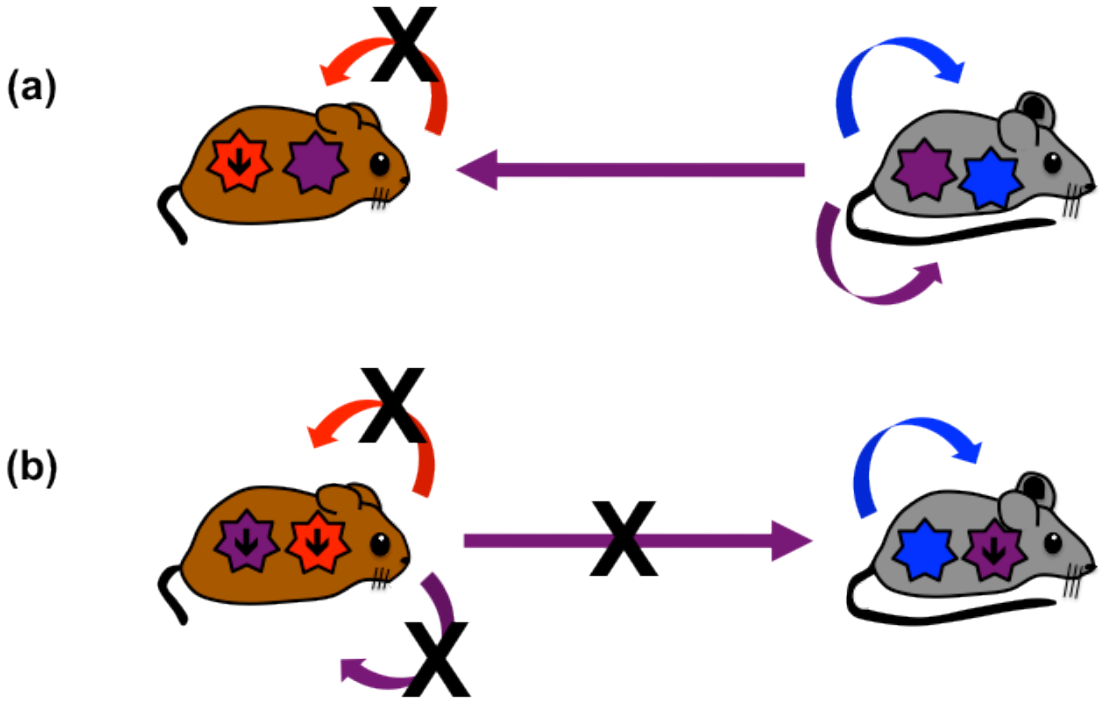

Figure 5.2: Predicted effects of Fipronil treatment on risk of *Bartonella* infection in bank voles and wood mice.
Figures of bank voles and wood mice represent populations of each host species respectively. Coloured stars represent *Bartonella* parasites. Red parasites are only transmitted between bank voles, blue parasites are only transmitted between wood mice, purple parasites are transmitted between wood mice and bank voles. 🡻 within a star indicates a reduction in infection risk as a result of Fipronil treatment of bank voles. Coloured arrows represent the direction of *Bartonella* transmission between host species. Black crosses indicate that a transmission route has been blocked as a result of Fipronil treatment of the bank vole population. There are two alternative scenarios regarding the effect of treatment on shared variants, depending on whether transmission of those variants is primarily mouse-to-vole or vole-to-mouse:
(a) Treatment of the bank vole population reduces risk of bank vole infection with those parasites that are transmitted only within the bank vole population (red). Risk of bank vole infection with host-shared *Bartonella* variants (purple) will persist if these parasites are transmitted from wood mice to bank voles. Risk of infection with all *Bartonella* species and variants in wood mice is unaffected (purple and blue).
(b) Treatment of the bank vole population reduces risk of bank vole infection with all *Bartonella* parasites. Risk of infection in wood mice with *Bartonella* parasites transmitted only within the wood mouse population is unaffected, but risk of infection with host-shared variants may be reduced if persistence requires transmission from bank voles.

5.2 Methods

5.2.1 Field methods

Sympatric populations of wood mice and bank voles were monitored longitudinally within three areas of mixed deciduous woodland in northwest England: Manor Wood (MW; N 53.3301°, E -3.0516°), Maresfield & Gordale woods (MFG; N 53.2729°, E -3.0615°) and Rode Hall (RH; N 53.1213°, E -2.2798°). Sherman live-traps (Alana Ecology, UK) were deployed in pairs at 10m intervals within three discrete trapping grids at each site (Figure 5.3). Most trapping grids measured 50m x 50m (72 traps per grid), with the exception of a single trapping grid at MW (MW1), which measured 70m x 70m (128 traps). Trapping sessions took place every four weeks from May to December in 2011 at MW and in 2012 at MFG and RH (eight trapping sessions at each site). At MFG and RH, all grids were trapped in each session and all grids were trapped at the same time within each site. At MW, trapping sessions on MW1 took place two weeks prior to trapping on MW2 and MW3 each session, and each grid was trapped for seven sessions only: MW1 was trapped from June to December, and MW2 and MW3 were trapped from May to November.

Upon first capture, all individuals were fitted with a sub-cutaneous electronic passive induced transponder (PIT-tag) (AVID MicroChips, UK), enabling identification of individuals when re-captured. When first captured within a monthly trapping session, individuals were checked for flea infestation and a small blood sample (~30µL) was taken from the tail tip for subsequent characterisation of *Bartonella* infections. Morphometric data were also taken. Full details of field methods and data collected are described elsewhere (Chapter 2).

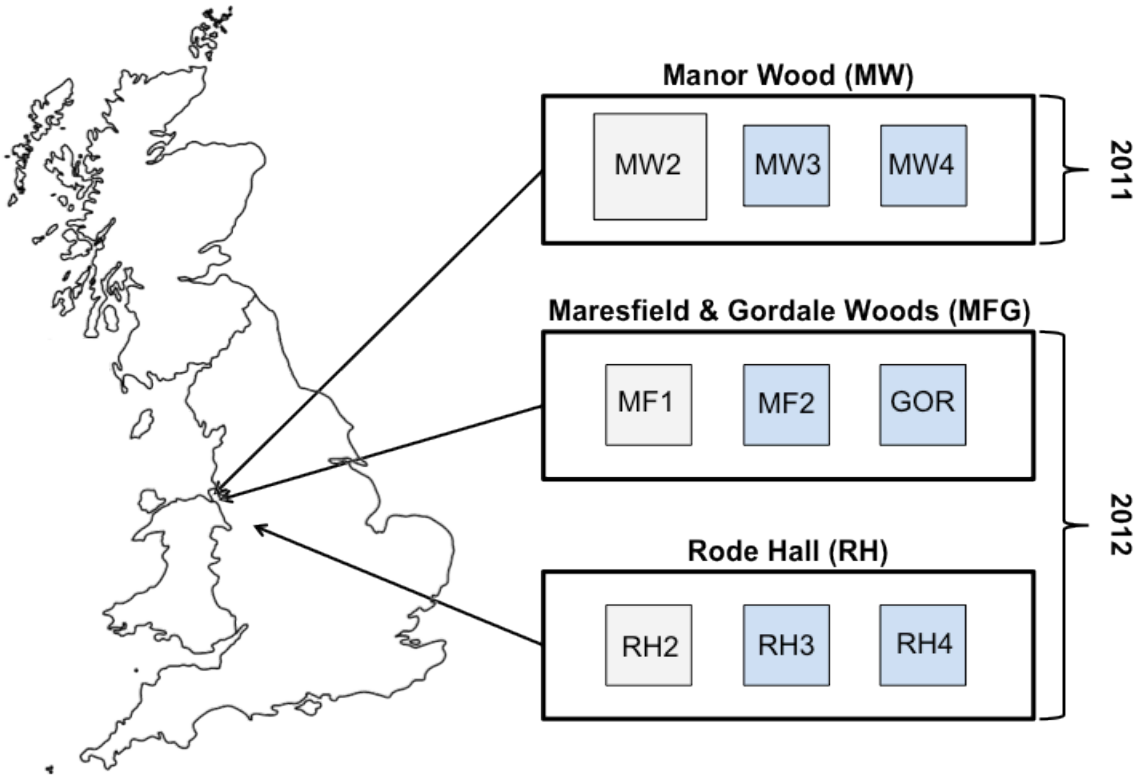

Figure 5.3: Locations of three woodland sites in northwest England in which rodents were captured during 2011 or 2012. Grey squares within each woodland rectangle refer to control grids at each site. Blue squares refer to treatment grids at each site. Most grids measured 50m x 50m except for MW2, which measured 70m x 70m.

5.2.2 Experimental design

Rodent communities within two grids at each site (Figure 5.3) were exposed to an experimental treatment that aimed to reduce the force of *Bartonella* spp. infection from bank voles. From July to December (sessions 3-8), all bank voles caught on these treatment grids were given a dermal weight-specific dose (10mg kg^{-1}) of the broad-spectrum veterinary insecticide Fipronil (Frontline Plus®) (Metzger & Rust, 2002) at first capture within a monthly trapping session (analogous to the methods of Smith *et al*., 2006). The liquid treatment was applied underneath the chin using a pipette to reduce the possibility that it would be removed by grooming. Fipronil is mildly toxic to small mammals (oral LD50 in mice = 95 mg kg^{-1} and dermal LD50 in rats >2000 mg kg^{-1}; Wiedemann, 2000), but a previous study found no effects of Fipronil treatment on the survival of wild field voles, *Microtus agrestis* (Smith *et al*., 2006). The third trapping grid at each site was an unmanipulated control grid (Figure 5.1). No wood mice on any grids ever received treatment.

Fipronil exerts largely insect-specific neurotoxic effects (Anadon & Gupta, 2012) and kills both adult and larval fleas within 24 hours of ingestion (Melhorn *et al*., 2001). As a result of this slight delay in treatment response, treated bank voles were still at risk of being bitten by fleas carrying *Bartonella* parasites contracted from other untreated bank voles or wood mice (Figure 5.1), therefore treatment is not expected to have affected the risk of an individual bank vole being infected with fleas or any *Bartonella* parasites. However, subsequent death of fleas contacting treated hosts should have prevented onwards transmission of any *Bartonella* parasites infecting the treated individual (Figure 5.1), and reduced the prevalence of bank vole-transmitted *Bartonella* parasites within the flea community.

5.2.2 Detection and characterisation of *Bartonella* infections

DNA was extracted from blood samples as detailed in Chapter 2, Section 2.2.2. Extractions were then used as templates in semi-nested polymerase chain reactions targeting a 300-500bp fragment of the *Bartonella* 16S-23S internal transcribed spacer (pITS) region, using the genus-specific primers of Telfer *et al*. (2005) (first round primers: *bigF* and *bogR*; second round primers: *bigF* and *bigR*). Full details of primer sequences, PCR reaction mixtures and thermal cycling programmes are given in Chapter 2, Section 2.2.2.

The infections of all positive samples were first identified to species by length polymorphism of the partial ITS (pITS) region targeted by the PCR, following the methods of Telfer *et al*. (2005, 2007), and detailed in Chapter 2, Section 2.2.2. Previous work (Chapter 3) has found that several species of *Bartonella* that appear to infect both wood mice and bank voles at these sites (*B. grahamii*, *B. taylorii* and *B. birtlesii*) comprise a complex of pITS variants, some of which are found in both hosts ("host-shared" variants) and others that have only been found in one ("host-exclusive" variants). The PCR products of a subset of samples positive for one of these three *Bartonella* species were also sequenced at this region (55% of *B. grahamii* infections, 59% of *B. taylorii* infections, and 27% of *B. birtlesii* infections). Samples were chosen from all *Bartonella* species infecting both host species at all sites, from across treatment and observation grids, and representing trapping sessions that occurred both before and after treatment of bank voles began. Sequencing methods followed those outlined in Chapter 3 (Section 3.2.2.2).

5.2.3 Data analysis

Population densities were calculated for each host species as the minimum number known alive (MNKA) on each trapping grid in each trapping session – a metric known to be highly correlated with other estimates of population density (Clotfelter *et al*., 2007). The number of bank voles treated in each session were summed, and the proportion of the bank vole community that were treated in each session on each grid

was calculated as the number of bank voles treated ÷ bank vole density. This density measure includes some individuals that were not captured in a particular session and therefore the proportion of bank voles treated was always <1. I also calculated the proportion of the total community (wood mice and bank voles combined) that were treated (number of bank voles treated ÷ total rodent MNKA). Due to changes in community composition over time, the proportion of the bank vole population and the proportion of the total rodent community that were treated varied independently.

The effect of Fipronil treatment on the risk of *Bartonella* infection in both host species was investigated using two approaches. The first sought to identify treatment effects based on species-level characterisation of *Bartonella* infections. The second approach investigated treatment effects based on pITS variant-level characterisation of *Bartonella* parasites. As *Bartonella* transmission is mediated by fleas, I also investigated the effect of bank vole treatment on the risk of flea infection in both host species.

5.2.3.1 The effect of bank vole treatment on risk of flea and *Bartonella* species infection

To assess whether bank vole treatment affected risk of flea and *Bartonella* species infection in either host species, I used a generalized linear mixed modelling (GLMM) approach (logit link, binomial errors). Infection risk of fleas (present or absent) and of each *Bartonella* species was modelled for wood mice and bank voles separately, and in each case, infection status of each sample in the data set was used as the binomial response (0 or 1). Fixed effects were investigated in two stages: First, individual age (young or adult), sex, reproductive condition (active or not active; see Chapter 2 for definition), and an interaction between sex and reproductive condition were assessed as intrinsic determinants of infection risk. For the models of infection risk in bank voles, I also included a 2-level factor indicating whether an individual had been treated with Fipronil in the previous trapping session. All models included animal ID number as a random effect, to account for potential pseudoreplication due to repeated sampling of the same individuals through time. Significant effects were identified by systematically removing the least significant term according to likelihood ratio tests and only retaining factors with a p-value <0.05. A sinusoidal term with 12-month periodicity (see Chapter

2) was included in all models to account for inherent seasonality of infection risk. Significant intrinsic risk factors identified in this first stage were then maintained in all subsequent models.

In the second stage of modelling, the effect of bank vole treatment on an individual's risk of infection was investigated. The degree to which treatment may have impacted *Bartonella* transmission within the community, and therefore impact individual infection risk, is likely to depend on the level of treatment (treatment coverage) imposed on the community (Figure 5.1). I therefore investigated whether treatment of the bank vole population, and the level of treatment applied on that grid, explained infection risk in each host species using the following three variables:

(1) The proportion of bank voles that were treated on the trapping grid two months previously.
(2) The proportion of the total rodent community (bank voles and wood mice combined) on the trapping grid that were treated two months previously.
(3) The cumulative number of bank voles that were treated at least once on the trapping grid up to two months previously.

Variables (1) and (2) investigated the possibility that a treatment effect was short-lived, such that the infectious flea population was reduced with increasing proportion of bank voles treated in the recent past. The proportion of the total community treated was investigated to account for the fact that both rodent species may contribute to the force of infection for some parasites, therefore a treatment effect may only be apparent if a large proportion of the total community was treated. Variable (3) was investigated because the effect of treatment may have built up over time, as more individuals were treated and therefore a larger proportion of the flea community were exposed to the effects of treatment.

The explanatory power of each of these treatment variables was investigated in separate models of infection risk. Each term was entered as a fixed effect in a GLMM along with the seasonal component and any significant intrinsic effects identified in stage one. Rodent population dynamics varied between woodland sites; it is therefore likely that baseline *Bartonella* transmission dynamics, and the magnitude of any treatment effects

may have also varied between woods. All models therefore also included 'wood' as a fixed effect, either as an additive term, or as an interaction with the treatment variable. A null model was also constructed, which included no treatment variables (i.e. only intrinsic variables, seasonality, and wood were included). Animal ID number was retained as a random effect in all models. Models were compared by their AICc values (Akaike Information Criterion, adjusted for small sample size; Anderson *et al*., 2000; Johnson & Omland, 2004), and the best model was identified as the one with the lowest AICc value. Models with an AICc value within 2 units of the model with the lowest AICc were considered equally likely to be the best model (Burnham & Anderson, 2002). If this situation arose, the most parsimonious model was considered the best (i.e. the model with fewest terms and no interactions). Evidence of a treatment effect was therefore confirmed if a model that included a treatment variable had an AICc value more than 2 units below that of the null model. A treatment effect was considered supported across all woodland sites unless a model that included an interaction between wood and a treatment variable had an AICc value more than 2 units below the corresponding treatment model that did not include the wood interaction. As there are limitations related to the comparison of non-nested mixed models using information criteria such as AICc (Bolker *et al*., 2009), I also ran all models in this second stage as GLMs without random effects, incorporating all intrinsic variables that were supported using the GLMM approach in the first stage of modelling. The results of the GLMs were in broad agreement with those of the GLMMs, therefore I present only the results of the GLMMs in the main text. Model selection tables, showing AICc comparisons for GLMs can be found in Appendix 5.6.2.

GLMs and GLMMs were fitted using Laplace approximation to maximum likelihood estimation, using R (Version 2.14.2). Mixed effects models were implemented in the *lme4* package in R, and fitted using the BOBYQA optimizer with a maximum of 20,000 iterations to aid convergence (github.com/lme4/lme4).

5.2.3.2 The effect of bank vole treatment on risk of host-exclusive and host-shared *Bartonella* pITS variants.

As the transmission of *Bartonella* between bank voles and wood mice may be limited to a few host-shared sub-specific variants, I also investigated the effect of treatment on the risk of infection with different genetic variants of each *Bartonella* species in each host species. However, as the prevalence of individual variants was often low in each host species, statistical analyses could not be performed as described above for each variant. Instead, the effect of bank vole treatment was investigated for *Bartonella* variants grouped as bank vole-exclusive, wood mouse-exclusive or host-shared, with variants across all *Bartonella* species combined. I categorised each *Bartonella* parasite that was sequenced at the partial ITS region (pITS) (n=796) into three groups, depending on whether it was a genetic variant found exclusively in wood mice, exclusively in bank voles, or in both host species, according to the results of Chapter 3. This categorisation was carried out for data within each site, such that a variant was classed as host-shared only if found in both host species at the same site. Furthermore, as some variants that were classified as host-shared had a highly heterogeneous distribution across host species, a variant was ultimately classed as host-shared only if >5% of all isolates within a site were detected in each host species. This reduced the likelihood of categorising a variant as host-shared if only very occasionally found in one of the host species, thus possibly representing a rare spillover transmission event (i.e. Types 03, 05 and 11; see Table 5.10). For each host species, I then investigated whether risk of infection with host-exclusive and host-shared *Bartonella* variants was affected by treatment, in an approach that followed the same format as outlined for the first analysis in Section 5.2.3.1. Individuals that tested positive for *Bartonella* infection but with an unknown genetic variant (because the parasite was not sequenced) were omitted from the data set.

5.3 Results

5.3.1 Rodent populations and treatment coverage

A total of 694 wood mice (1827 captures) and 691 bank voles (1547 captures) were trapped across the three woodland sites (Table 5.1). Seasonal variation in population densities differed between rodent species, with bank voles generally displaying an earlier peak in density compared to wood mice, across all sites (Figure 5.4). There were also differences in seasonal variation and relative densities of wood mice and bank voles between sites. In particular, densities of bank voles were markedly higher than wood mice at MW for much of the year (Figure 5.4a-c). In contrast, wood mice dominated communities at MFG (Figure 5.4d-f), and species at RH were more evenly represented (Figure 5.4g-i).

Table 5.1: Number of individuals and total number of captures of each rodent species at each site.

	Manor Wood		**Maresfield & Gordale**		**Rode Hall**	
	WM	**BV**	**WM**	**BV**	**WM**	**BV**
# individuals	226	307	284	189	184	195
# captures	537	665	758	454	532	428

Following the onset of treatment in July, the cumulative number of individuals treated at least once gradually increased over time to a plateau on all six treatment grids (Figure 5.4a-c). The proportion of bank voles treated each session (number treated ÷ MNKA) fluctuated during the post-treatment period on all treatment grids between 0.19 – 0.83 at MW (Figure 5.5d), 0.20 – 0.87 at MFG (Figure 5.5e) and 0.40 – 0.86 at RH (Figure 5.5f). Similarly, the proportion of the total community treated fluctuated on all treatment grids during the post-treatment period between 0.06 – 0.59 at MW (Figure 5.5g), 0.04 – 0.46 at MFG (Figure 5.5h) and 0.17 – 0.63 at RH (Figure 5.5i). There was a notable decline in the proportion of the total community treated from July to December at all sites, as (untreated) wood mice came to dominate rodent communities towards the end of the year.

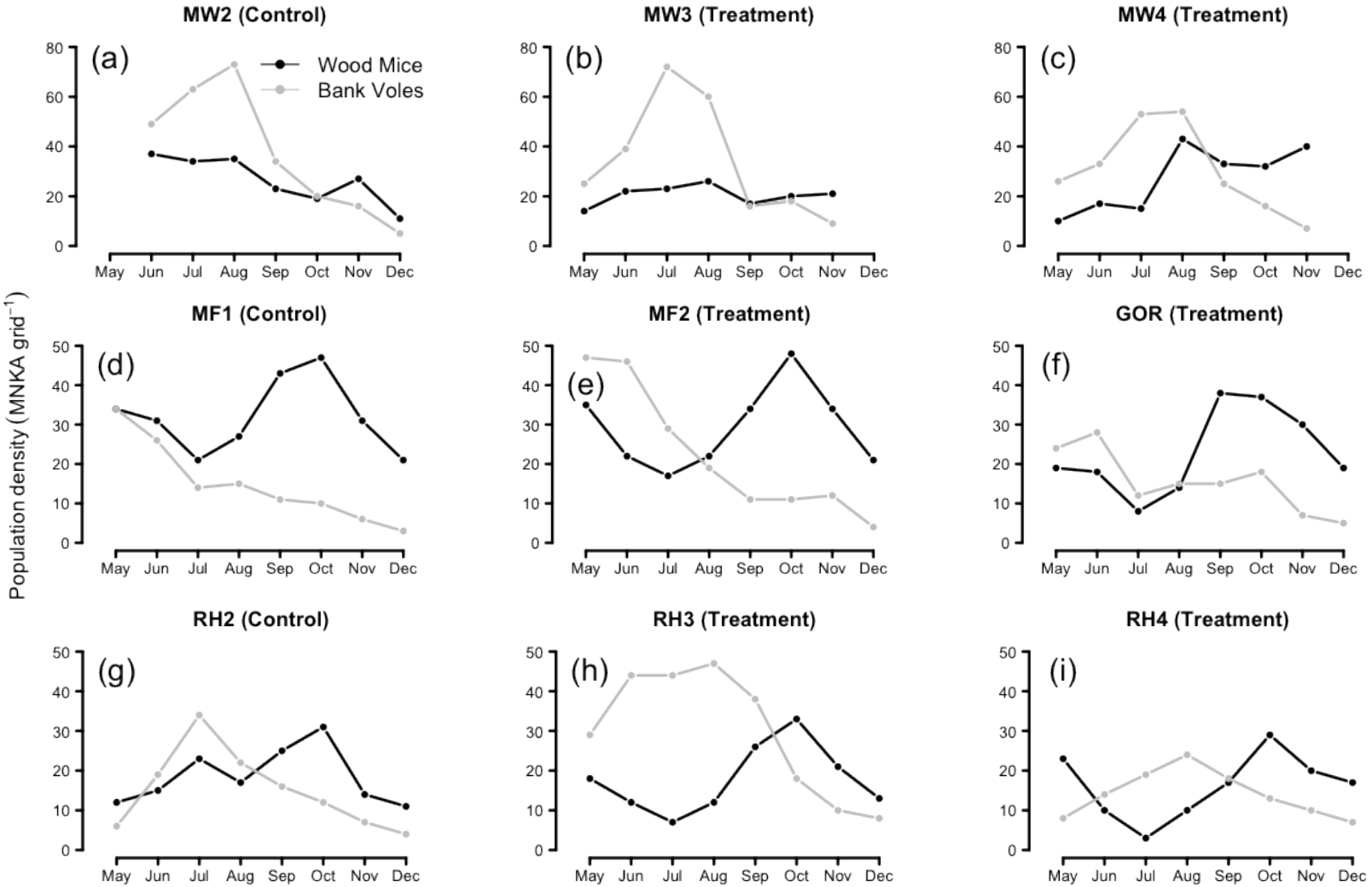

Figure 5.4: Population densities (minimum number known alive; MNKA) of wood mice (black lines) and bank voles (grey lines) on each of three grids at Manor Wood (top row), Maresfield & Gordale (middle row) and Rode Hall (bottom row).

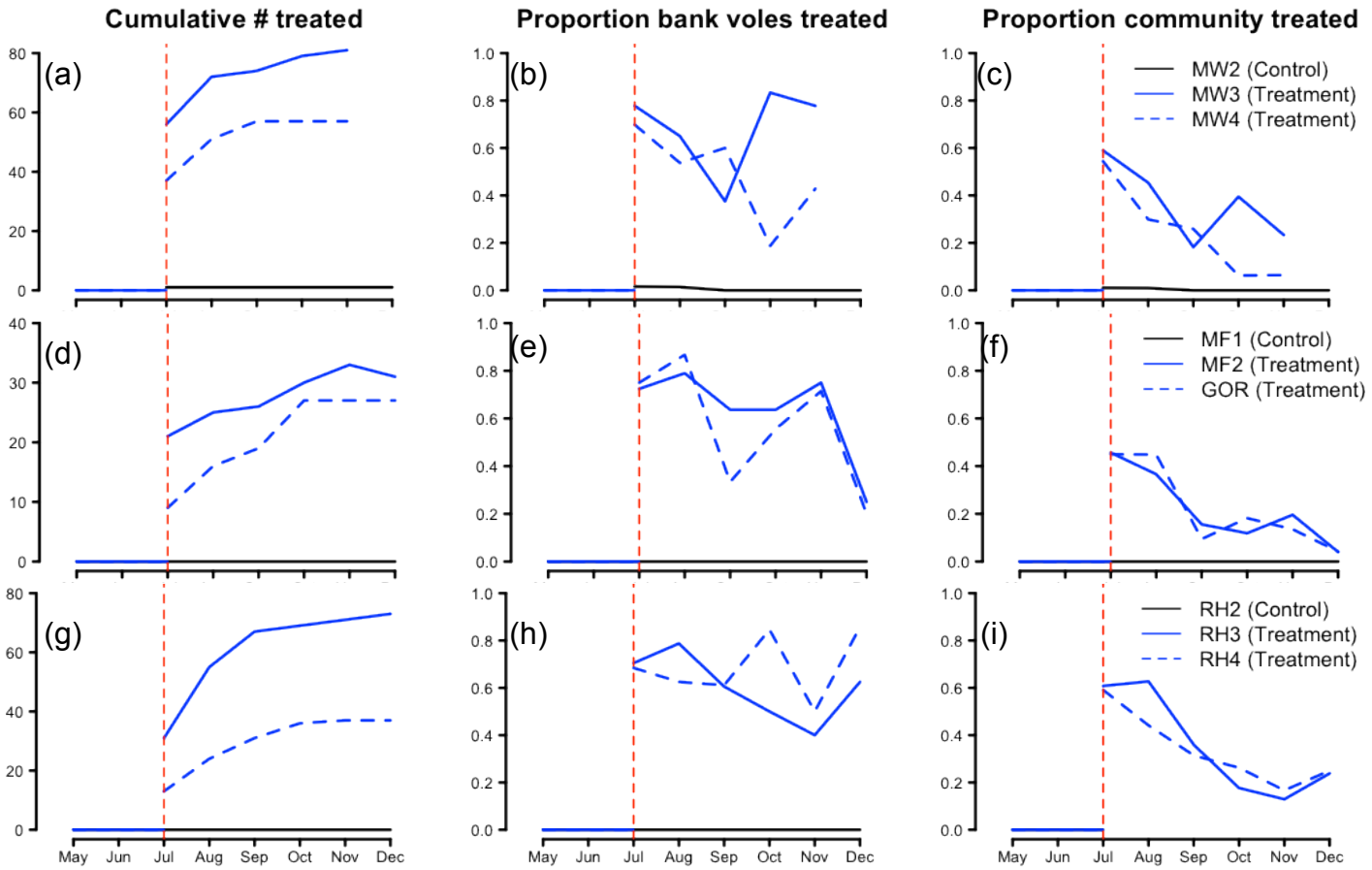

Figure 5.5: Cumulative number of bank voles treated, proportion of the bank vole population treated and proportion of the total rodent community treated on each grid at Manor Wood (a-c), Maresfield and Gordale (d-f) and Rode Hall (g-i). Blue lines = treatment grids; black lines = control grids. Red dashed lines indicate when Fipronil treatment of bank voles began.

5.3.2 Flea infections

Fleas were found to infect a greater proportion of the bank vole population compared to the wood mouse population at all three sites (Table 5.2). At MW and MFG, there was generally greater prevalence of flea infection in both bank voles (Figure 5.6a-b) and wood mice (Figure 5.6d-e) earlier in the trapping season. In contrast, flea prevalence at RH saw a general increase in bank voles later in the year (Figure 5.6c), and was comparatively low throughout the year in wood mice (Figure 5.6f).

Table 5.2: The proportion (and number) of individuals infected with fleas at least once at each site.

Manor Wood		**Maresfield & Gordale**		**Rode Hall**	
WM ***n*** **= 226**	**BV** ***n*** **= 307**	**WM** ***n*** **= 284**	**BV** ***n*** **= 189**	**WM** ***n*** **= 184**	**BV** ***n*** **= 195**
0.11 (*n*=24)	0.23 (*n*=72)	0.20 (*n*=57)	0.24 (*n*=46)	0.16 (*n*=29)	0.24 (*n*=46)

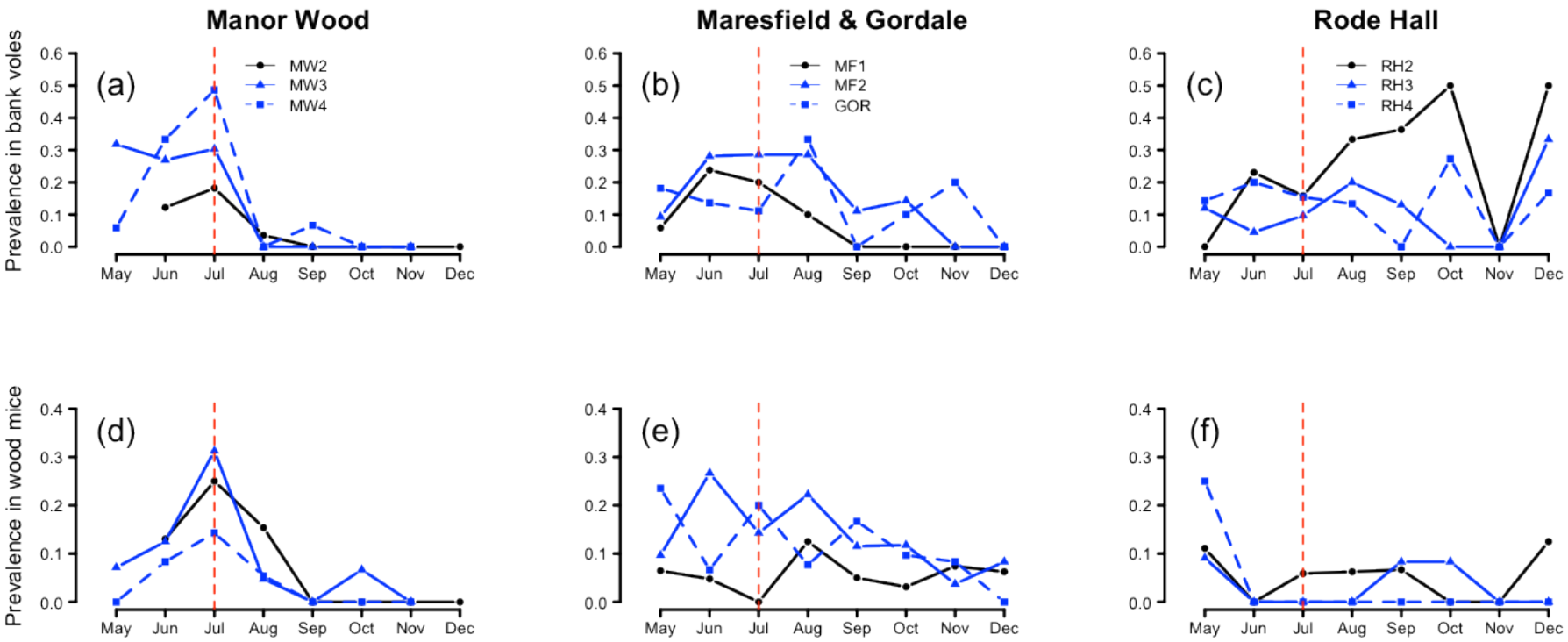

Figure 5.6: Prevalence of flea infection throughout the study period in bank voles (a-c) and wood mice (d-f) on each of three grids at each woodland site. Blue lines = treatment grids; black lines = control grids. Red dashed lines indicate when Fipronil treatment of bank voles began.

5.3.2.1 Models of flea infection risk

Bank Voles

Reproductive condition was identified as a contributor to risk of flea infection in bank voles (χ^2=4.05, df=1, p=0.04) and therefore included in all subsequent models. Infection risk was not affected by whether an individual was treated in the previous session (χ^2=3.38, df=1, p=0.07). No models of flea infection risk that included treatment effects were supported (Table 5.3). The parameter estimates for all fixed effects within the best model of flea infection risk in bank voles (and all other host-parasite combinations that follow) are given in Appendix 5.6.1.

Wood Mice

No individual characteristics were identified as significant contributors to infection risk. Two models that included treatment variables were equally supported (Table 5.3). One included an effect of the proportion of bank voles previously treated, and the other included an effect of the proportion of the total community previously treated. In both cases, the treatment effects were only supported when an interaction with wood was included, indicating that the effect of treatment differed between woods (Table 5.3). Risk of flea infection in wood mice increased at MFG and decreased at MW with increasing proportion of the vole population (Figure 5.7a) or the total rodent community (Figure 5.7b) treated. There was no effect at RH. Note that one of the candidate models failed to converge for wood mouse infection risk (model #2, Table 5.3). However, the same models were identified as best when GLMs were used and AICc values were available for all models (Appendix 5.6.2).

Table 5.3: Model selection table for risk of flea infection in bank voles and wood mice. All models included a sinusoidal seasonal term and wood as fixed effects. All bank vole models also included reproductive condition as a fixed effect. AICc for each model is given. The best model (the most parsimonious model(s) with lowest AICc) is highlighted in yellow. 'DNC' = model did not converge.

Model No.	Model	AICc	
		Bank Voles	Wood Mice
1.	Season + Wood	906.6	610.5
2.	Season + Wood + Cumulative # treated	906.3	DNC
3.	Season + Wood + Proportion of bank voles treated	908.5	611.3
4.	Season + Wood + Proportion of rodent community treated	909.6	611.7
5.	Season + Wood x Cumulative # treated	908.6	609.6
6.	Season + Wood x Proportion of bank voles treated	908.6	607.5
7.	Season + Wood x Proportion of rodent community treated	908.6	608.0

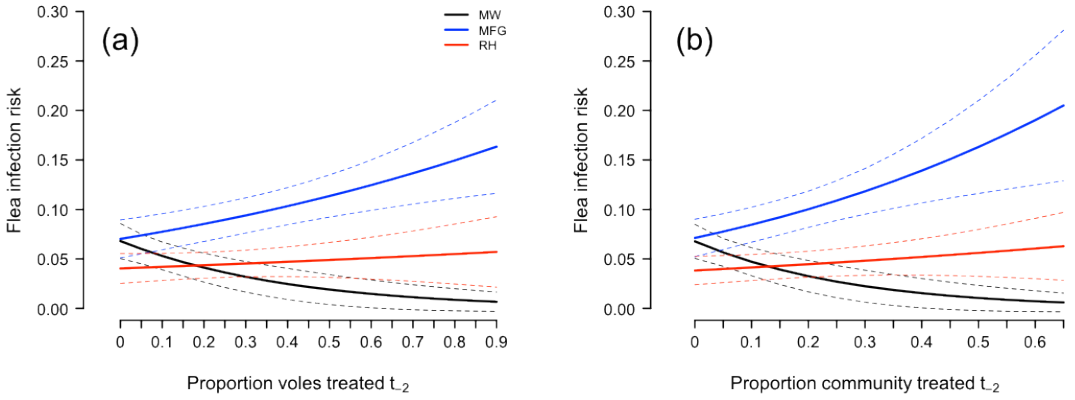

Figure 5.7: Predicted effect of Fipronil treatment of bank voles on flea infection risk in wood mice (dashed lines show standard errors). Two models that included different treatment effects were equally supported: (a) proportion of bank voles treated two months previously and (b) proportion of the total rodent community treated two months previously. Both models indicated different effects of treatment at the three sites. An increase in the proportion of bank voles or the total community that were treated previously saw a decrease in flea infection risk at MW (black lines), an increase at MFG (blue lines), and had no effect at RH (red lines). The models also included a sinusoidal seasonality term. These predictions relate to infection risk in September.

5.3.3 *Bartonella* spp. infections and models of infection risk

Seven species of *Bartonella* were detected within rodent blood samples (Table 5.4; Figure 5.8 and Figure 5.9). *Bartonella grahamii*, *B. taylorii* and *B. birtlesii* were found in both wood mice and bank voles, although *B. birtlesii* was not present in bank voles at RH (Figure 5.9i). In contrast, *B. rudakovii* and *B. doshiae* were found only in bank voles and *B. doshiae*-like and BGA only in wood mice, at all sites (Table 5.4). Prevalence of infection was consistently low (≤10%) across two of the three sites for BGA in wood mice (Figure 5.9j-k) and across all sites for *B. doshiae* in bank voles (Figure 5.8m-o), and for *B. grahamii* in wood mice at RH (Figure 5.9c) and *B. taylorii* in bank voles at MFG (Figure 5.8e). These host-parasite-site combinations were therefore omitted from the following analyses.

Table 5.4: Proportion (and number) of individuals infected at least once with a *Bartonella* species at each site. Cells in grey relate to *Bartonella* species that infected ≤10% of a host species at a given site, and were therefore not included in any models of infection risk.

***Bartonella* species**	**Manor Wood**		**Maresfield & Gordale**		**Rode Hall**	
	WM *n* = 226	**BV *n* = 307**	**WM *n* = 284**	**BV *n* = 189**	**WM *n* = 184**	**BV *n* = 195**
B. grahamii	0.12 (*n*=28)	0.33 (*n*=101)	0.11 (*n*=32)	0.12 (*n*=23)	0.03 (*n*=6)	0.13 (*n*=26)
B. taylorii	0.33 (*n*=74)	0.17 (*n*=51)	0.14 (*n*=41)	0.10 (*n*=18)	0.41 (*n*=75)	0.36 (*n*=71)
B. birtlesii	0.19 (*n*=43)	0.20 (*n*=62)	0.45 (*n*=128)	0.18 (*n*=34)	0.40 (*n*=72)	0
B. rudakovii	0	0.11 (*n*=34)	0	0.19 (*n*=35)	0	0.21 (*n*=40)
B. doshiae	0	0.02 (*n*=6)	0	0.02 (*n*=4)	0	0.01 (*n*=2)
BGA	0.02 (*n*=5)	0	0.05 (*n*=14)	0	0.10 (*n*=19)	0
B. doshiae-like	0.15 (*n*=33)	0	0.05 (*n*=14)	0	0.15 (*n*=27)	0

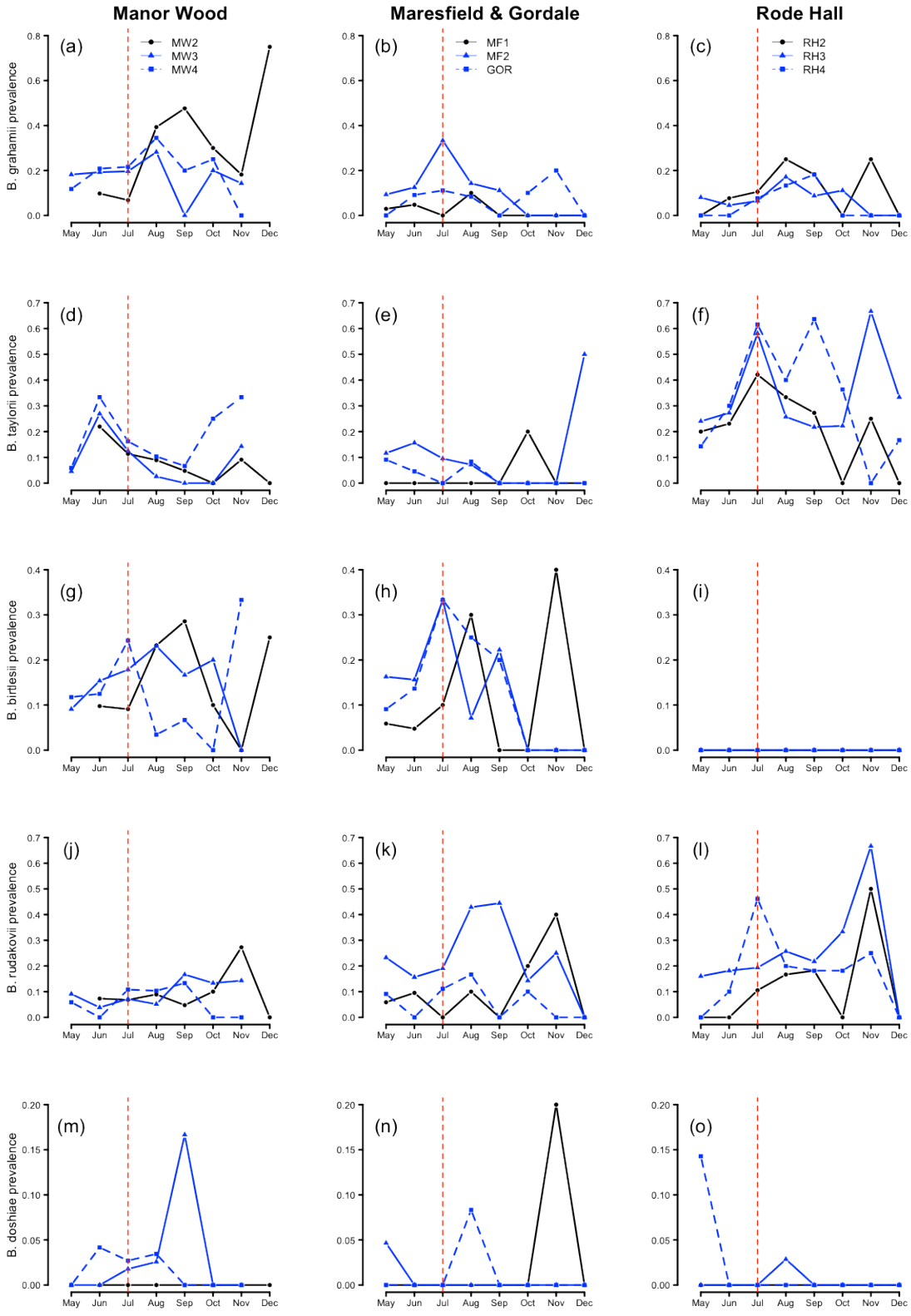

Figure 5.8: Prevalence of the five *Bartonella* species infecting bank voles on each of three grids at each site. (a-c) *B. grahamii* (d-f) *B. taylorii* (g-i) *B. birtlesii* (j-l) *B. rudakovii* (m-o) *B. doshiae*. Blue lines = treatment grids; black lines = control grids; red dashed lines indicate when Fipronil treatment of bank voles began.

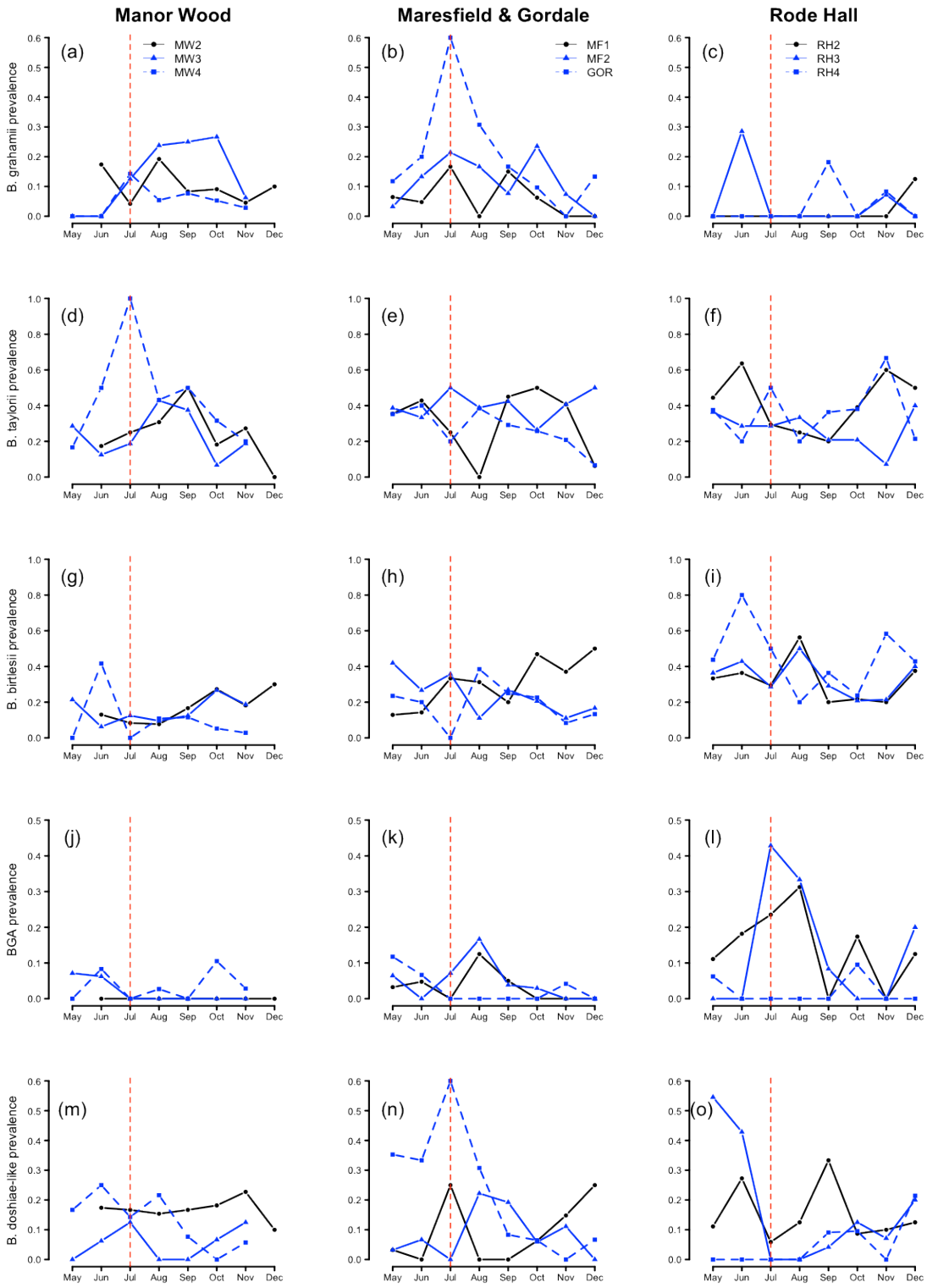

Figure 5.9: Prevalence of the five *Bartonella* species infecting wood mice on each of three grids at each site. (a-c) *B. grahamii* (d-f) *B. taylorii* (g-i) *B. birtlesii* (j-l) BGA (m-o) *B. doshiae*-like. Blue lines = treatment grids; black lines = control grids; red dashed lines indicate when Fipronil treatment of bank voles began.

5.3.3.1 Models of *B. grahamii* infection risk

Bank Voles

Age was identified as a significant contributor to *B. grahamii* infection risk in bank voles (χ^2=8.12, df=1, p=0.004) and individuals who were treated in the previous trapping session were also less likely to be infected with *B. grahamii* compared to those who were not treated (χ^2=7.22, df=1, p=0.007). These effects were maintained in all subsequent models. Two models of infection risk that included treatment variables were equally supported (Table 5.5). One included a negative effect of the cumulative number of bank voles treated previously, and the other included a negative effect of the proportion of bank voles treated previously (Figure 5.10). Interactions between these treatment variables and wood were not supported, indicating that the effects were consistent across all three sites.

Wood Mice

There was no support for any intrinsic effects on *B. grahamii* infection risk in wood mice, and models that included treatment variables were not supported. Note that two of the candidate models failed to converge for wood mouse infection risk (models #2 and #7, Table 5.5). However, the same model was identified as best when GLMs were used and AICc values were available for all models (Appendix 5.6.2).

Table 5.5: Model selection table for risk of *B. grahamii* infection in bank voles and wood mice. All models included a sinusoidal seasonal term and wood as fixed effects. All bank vole models also included age and treatment in the previous session as a fixed effect. Models of wood mouse infection risk did not include data from RH due to low prevalence at this site. AICc for each model is given. The best model (the most parsimonious model with lowest AICc) is highlighted in yellow. 'DNC' = model did not converge.

Model No.	Model	AICc	
		Bank Voles	Wood Mice
1.	Season + Wood	852.8	486.7
2.	Season + Wood + Cumulative # treated	849.1	DNC
3.	Season + Wood + Proportion of bank voles treated	847.8	488.5
4.	Season + Wood + Proportion of rodent community treated	850.4	488.6
5.	Season + Wood x Cumulative # treated	851.3	490.1
6.	Season + Wood x Proportion of bank voles treated	851.4	490.2
7.	Season + Wood x Proportion of rodent community treated	853.5	DNC

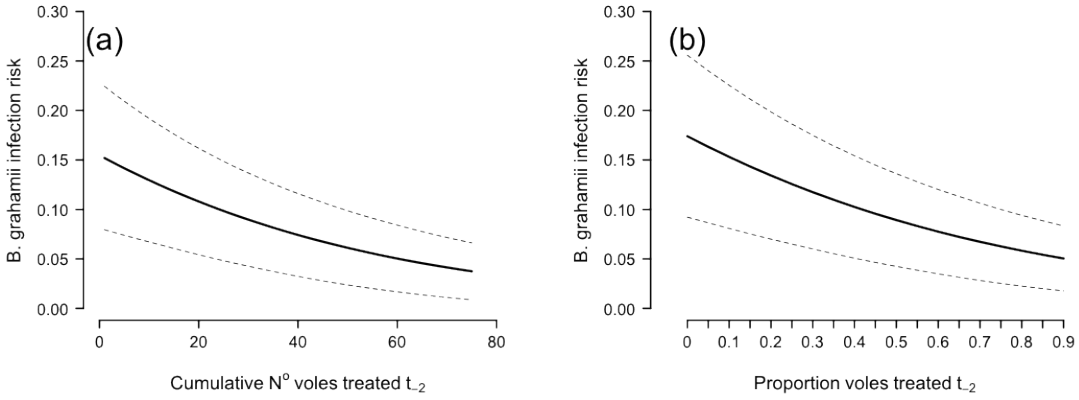

Figure 5.10: Predicted effect of Fipronil treatment of bank voles on *B. grahamii* infection risk in bank voles (dashed lines show standard errors). Two models that included different treatment effects were equally supported: (a) cumulative number of bank voles treated two months previously and (b) proportion of the bank vole population treated two months previously. Risk of infection was negatively associated with each of these variables. These effects were consistent across all sites. The models also included a sinusoidal seasonality term, wood, individual age and treatment in the previous month as fixed effects. The prediction here is shown for previously treated adult bank voles at MW in September.

5.3.3.2 Models of *B. taylorii* infection risk

Bank Voles

There was no support for any intrinsic effects on infection risk. Models that included treatment variables were not supported (Table 5.6).

Wood Mice

An interaction between individual sex and reproductive status was identified as a significant contributor to infection risk of *B. taylorii* in wood mice (χ^2=5.41, df=1, p=0.02), and so was age (χ^2=14.65, df=1, p<0.001). However, models that included treatment effects were not supported (Table 5.6). Note that one of the candidate models failed to converge for wood mouse infection risk (model #5, Table 5.5). However, the same model was identified as best when GLMs were used and AICc values were available for all models (Appendix 5.6.2).

Table 5.6: Model selection table for risk of *B. taylorii* infection in bank voles and wood mice. All models included a sinusoidal seasonal term and wood as fixed effects. All bank vole models also included treatment last session as a fixed effect. All wood mouse models also included age and an interaction between sex and reproductive condition as fixed effects. Models of bank vole infection risk did not include data from MFG due to low prevalence at this site. AICc for each model is given. The best model (the most parsimonious model with lowest AICc) is highlighted in yellow. 'DNC' = model did not converge.

Model No.	Model	AICc	
		Bank Voles	Wood Mice
1.	Season + Wood	710.8	1427.6
2.	Season + Wood + Cumulative # treated	710.0	1425.9
3.	Season + Wood + Proportion of bank voles treated	710.8	1428.5
4.	Season + Wood + Proportion of rodent community treated	711.7	1428.5
5.	Season + Wood x Cumulative # treated	711.6	DNC
6.	Season + Wood x Proportion of bank voles treated	712.5	1432.1
7.	Season + Wood x Proportion of rodent community treated	713.0	1431.2

5.3.3.3 Models of *B. birtlesii* infection risk

Bank Voles

There was no support for any intrinsic effects on infection risk, and no model that included a treatment effect was supported (Table 5.7).

Wood Mice

Age was identified as a significant contributor to infection risk of *B. birtlesii* in wood mice (χ^2=13.52, df=1, p<0.001). There was support for a model that included an effect of the cumulative number of bank voles treated previously (Table 5.7). This treatment effect was only supported when included as an interaction with wood, indicating that the effect of treatment differed between woods. There was a negative effect of the cumulative number of bank voles treated previously on infection risk in wood mice at MFG and RH, but no significant effect at MW (Figure 5.11).

Table 5.7: Model selection table for risk of *B. birtlesii* infection in bank voles and wood mice. All models included a sinusoidal seasonal term and wood as fixed effects. All wood mouse models also included age as fixed effects. Models of bank vole infection risk did not include data from RH, as *B. birtlesii* was absent from this site. AICc for each model is given. The best model (the most parsimonious model with lowest AICc) is highlighted in yellow.

Model No.	Model	AICc	
		Bank Voles	Wood Mice
1.	Season + Wood	573.7	1194.4
2.	Season + Wood + Cumulative # treated	575.4	1192.7
3.	Season + Wood + Proportion of bank voles treated	574.7	1192.9
4.	Season + Wood + Proportion of rodent community treated	574.9	1195.0
5.	Season + Wood x Cumulative # treated	576.4	1191.2
6.	Season + Wood x Proportion of bank voles treated	576.6	1196.8
7.	Season + Wood x Proportion of rodent community treated	576.9	1198.5

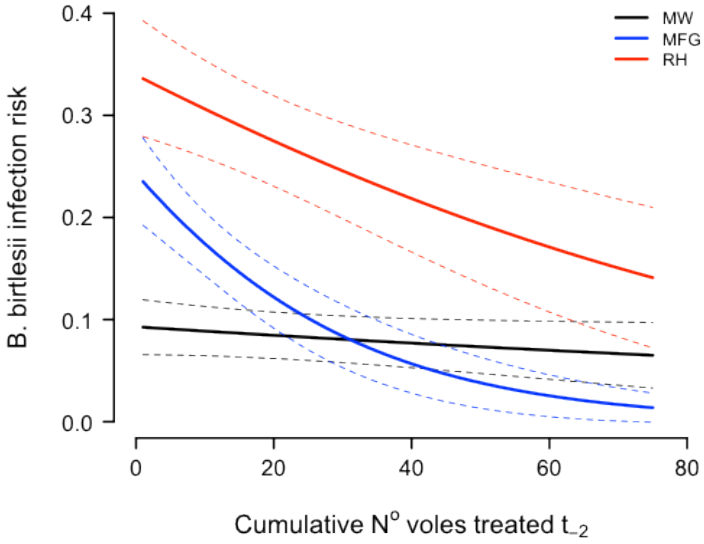

Figure 5.11: Predicted effect of Fipronil treatment on *B. birtlesii* infection risk in wood mice (dashed lines show standard errors). The model indicated different effects of treatment at the three sites. An increase in the cumulative number of bank voles treated up to two months previously saw a decrease in *B. birtlesii* infection risk at MFG, but there was no significant effect at MW or RH. The model also included a sinusoidal seasonality term and individual age as fixed effects. These predictions relate to infection risk of adults in September.

5.3.3.4 Models of *B. doshiae*-like infection risk (wood mice only)

Age (χ^2=7.71, df=1, p=0.006) was identified as a significant contributor to risk of *B. doshiae*-like infection in wood mice and this factor was included in all subsequent models. Models that included treatment effects were not supported (Table 5.8). Note that one of the candidate models failed to converge (model #6, Table 5.8). However, the same model was identified as best when GLMs were used and AICc values were available for all models (Appendix 5.6.2).

Table 5.8: Model selection table for risk of *B. doshiae*-like infection in wood mice. All models included a sinusoidal seasonal term, wood, age and reproductive condition as fixed effects. AICc for each model is given. The best model (the most parsimonious model with lowest AICc) is highlighted in yellow. 'DNC' = model did not converge.

Model No.	Model	AICc
1.	Season + Wood	624.8
2.	Season + Wood + Cumulative # treated	626.8
3.	Season + Wood + Proportion of bank voles treated	625.8
4.	Season + Wood + Proportion of rodent community treated	625.3
5.	Season + Wood x Cumulative # treated	630.4
6.	Season + Wood x Proportion of bank voles treated	DNC
7.	Season + Wood x Proportion of rodent community treated	627.8

5.3.3.5 Models of *B. rudakovii* infection risk (bank voles only)

There was no support for any intrinsic effects on infection risk, and there was no support for any models that included treatment effects (Table 5.9).

Table 5.9: Model selection table for risk of *B. rudakovii* infection in bank voles. All models included a sinusoidal seasonal term, wood, age and treatment last session as fixed effects. AICc for each model is given. The best model (the most parsimonious model with lowest AICc) is highlighted in yellow.

Model No.	Model	AICc
1.	Season + Wood	701.0
2.	Season + Wood + Cumulative # treated	702.9
3.	Season + Wood + Proportion of bank voles treated	702.5
4.	Season + Wood + Proportion of rodent community treated	703.0
5.	Season + Wood x Cumulative # treated	DNC
6.	Season + Wood x Proportion of bank voles treated	702.5
7.	Season + Wood x Proportion of rodent community treated	703.6

5.3.4 Genetic characterisation of *Bartonella* infections at the ITS region

In total, *Bartonella* parasites were sequenced at the pITS region from 303 rodents from MW, 273 rodents from MFG and 220 rodents from RH (Table 5.10). Four *Bartonella* species were each represented by a single pITS variant and each was only found in one host species; *B. doshiae*-like (Type 12) and BGA (Type 24) were both wood mouse-exclusive, and *B. doshiae* and *B. rudakovii* were both bank vole-exclusive. Of the apparently generalist *Bartonella* species, four *B. grahamii* variants (Types 02, 04, 09 and 10), eight *B. taylorii* variants (Types 05, 11, 13, 14, 16, 20, 21 and 29) and seven *B. birtlesii* variants (Types 03, 15, 17, 22, 23, 25 and 26) were identified, according to the nomenclature of Chapter 3, representing a range of host-exclusive and host-shared types (Table 5.10). Six host-shared variants were identified across the three sites, but two of these (Type 05 and Type 03) were classed as BV-exclusive and one (Type 11) was classed as WM-exclusive under the more conservative categorisation criteria.

Seasonal patterns of prevalence for each pITS variant of *B. grahamii*, *B. taylorii* and *B. birtlesii* are shown in Table 5.11, Table 5.12 and Table 5.13 respectively, for wood mouse and bank vole populations on the observation and two treatment grids (combined) at the three woodland sites. Note that only variants that were detected on more than a single occasion are presented in these figures. Variants are divided into those that are bank vole-exclusive, wood mouse-exclusive and host-shared. Some variants were found on treatment grids but not observation grids at the same site, and prevalence of all variants was often low in both host species, therefore effects of treatment were difficult to assess for individual variants from these plots of raw prevalence, and statistical analyses lacked power.

Table 5.10: Number of pITS variants of each *Bartonella* species found in each host species at each wood, classified into bank vole-exclusive (green), wood mouse-exclusive (red) or host-shared (purple). * indicates that fewer than 5% of isolates of this "host-shared" variant was found in either wood mice (Type 11) or bank voles (Type 03 and Type 05) at this site, and so these variants were reclassified as bank vole- or wood mouse-exclusive variants respectively. The numbers of host-exclusive and host-shared variants found at each site are given for categorisation criteria that were strict (host-shared if ever found in both host species) and more conservative (host-shared if >5% of the total number of this variant detected were found in each host species; numbers in brackets).

		MW		MFG		RH	
***Bartonella* species**	**pITS type (see Ch.3)**	**Wood Mice**	**Bank Voles**	**Wood Mice**	**Bank Voles**	**Wood Mice**	**Bank Voles**
***B. doshiae*-like**	Type 12	**15**		**23**		**15**	
BGA	Type 24	**6**		**11**		**17**	
B. doshiae	Type 01		**1**				**1**
B. rudakovii	Type 08		**37**		**15**		**16**
B. grahamii	Type 02		**76**		**18**		
	Type 04						**23**
	Type 09			**7**	**3**		
	Type 10	**19**	**6**	**25**	**5**	**3**	
B. taylorii	Type 05	**6**	**39**	**3**	**7**	**2***	**72***
	Type 11	**25**		**30***	**1***	**18**	
	Type 14			**15**		**1**	**3**
	Type 13			**6**			
	Type 16			**1**			
	Type 20	**20**		**46**		**31**	
	Type 21	**4**		**3**			
	Type 29	**1**		**5**			
B. birtlesii	Type 03		**24**	**1***	**19***	**1**	
	Type 15			**12**			
	Type 17			**8**			
	Type 22	**16**					
	Type 23	**6**		**9**		**17**	
	Type 25	**1**					
	Type 26	**1**					
#BV-exclusive variants		138		33 (52)		40 (112)	
#WM-exclusive variants		95		139 (169)		102 (102)	
#host-shared variants		25WM; 45BV		66(35)WM; 35(15)BV		3(1)WM; 75(3)BV	

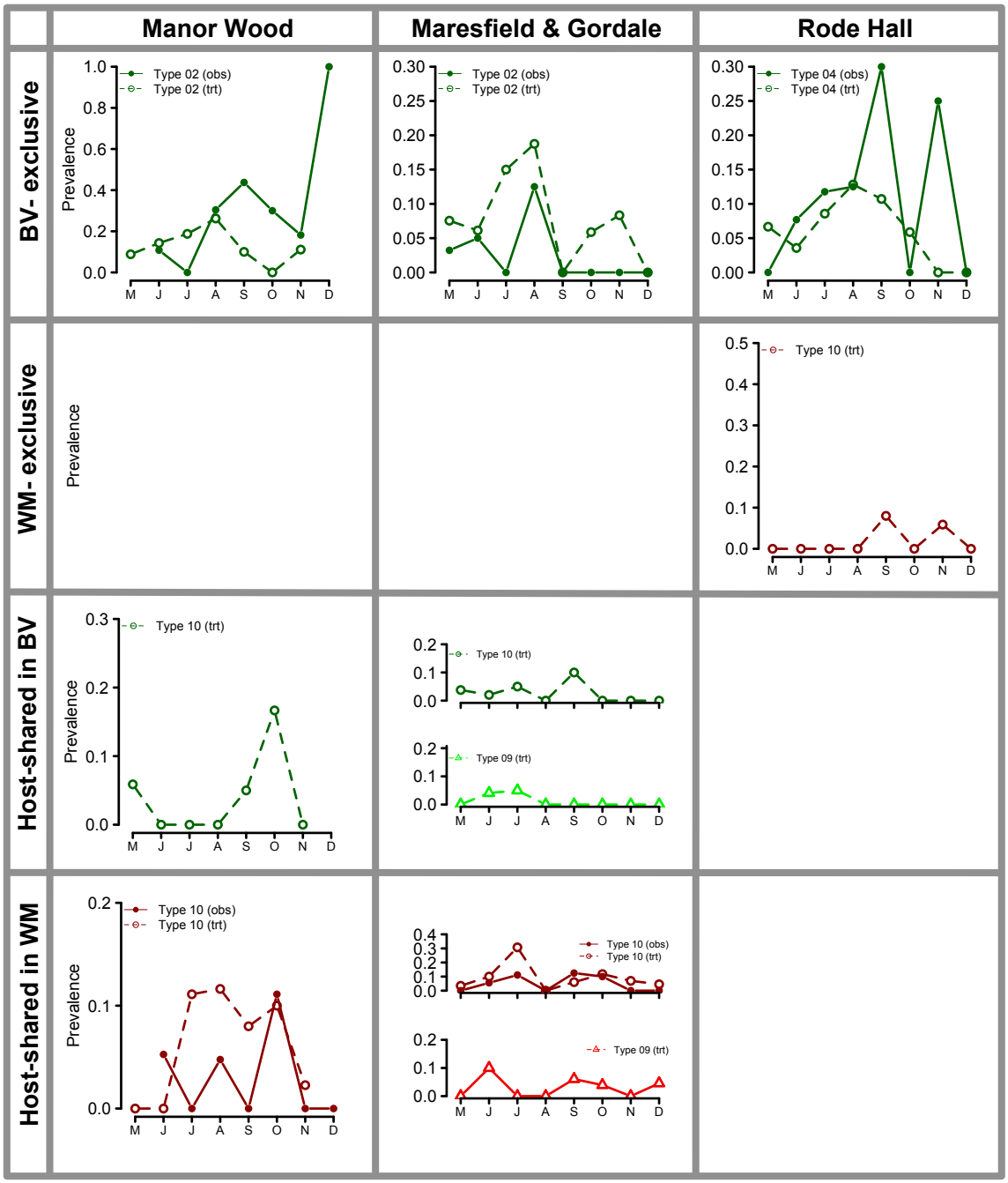

Figure 5.12: Seasonal prevalence of pITS variants of ***B. grahamii*** in wood mice and bank voles on observation and treatment grids at the three woodland sites. Letters on x-axes represent months of the year from May to December. Variants are divided into those that are bank-vole exclusive, wood mouse-exclusive and host-shared. Plots in green relate to prevalence in bank voles; plots in red relate to prevalence in wood mice. Estimates of prevalence omit samples that were positive for infection but not sequenced. The prevalences here are therefore likely to be underestimates.

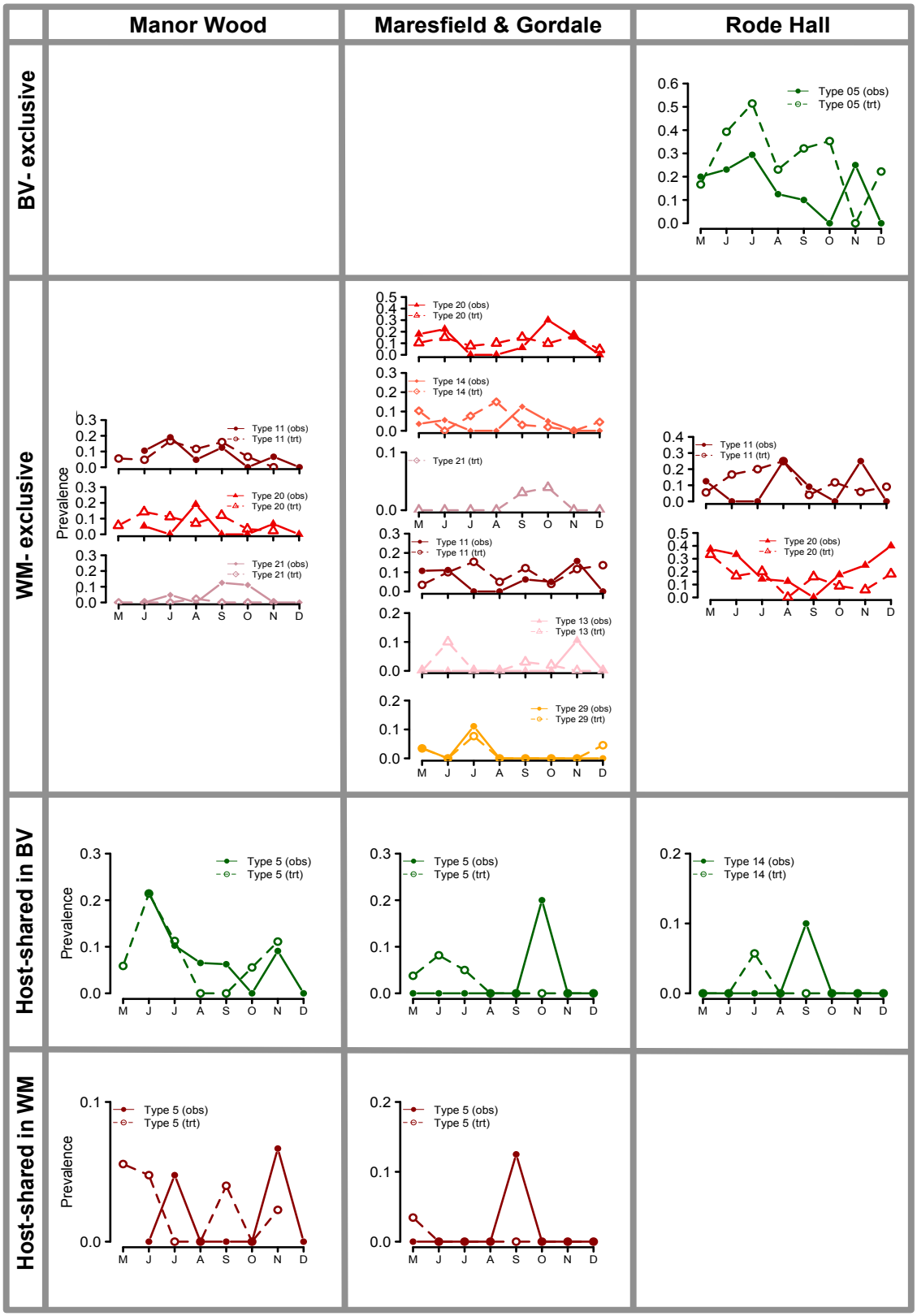

Figure 5.13: Seasonal prevalence of pITS variants of ***B. taylorii*** in wood mice and bank voles on observation and treatment grids at the three woodland sites. Letters on x-axes represent months of the year from May to December. Variants are divided into those that are bank-vole exclusive, wood mouse-exclusive and host-shared. Plots in green relate to prevalence in bank voles; plots in red relate to prevalence in wood mice. Estimates of prevalence omit samples that were positive for infection but not sequenced. The prevalences here are therefore likely to be underestimates.

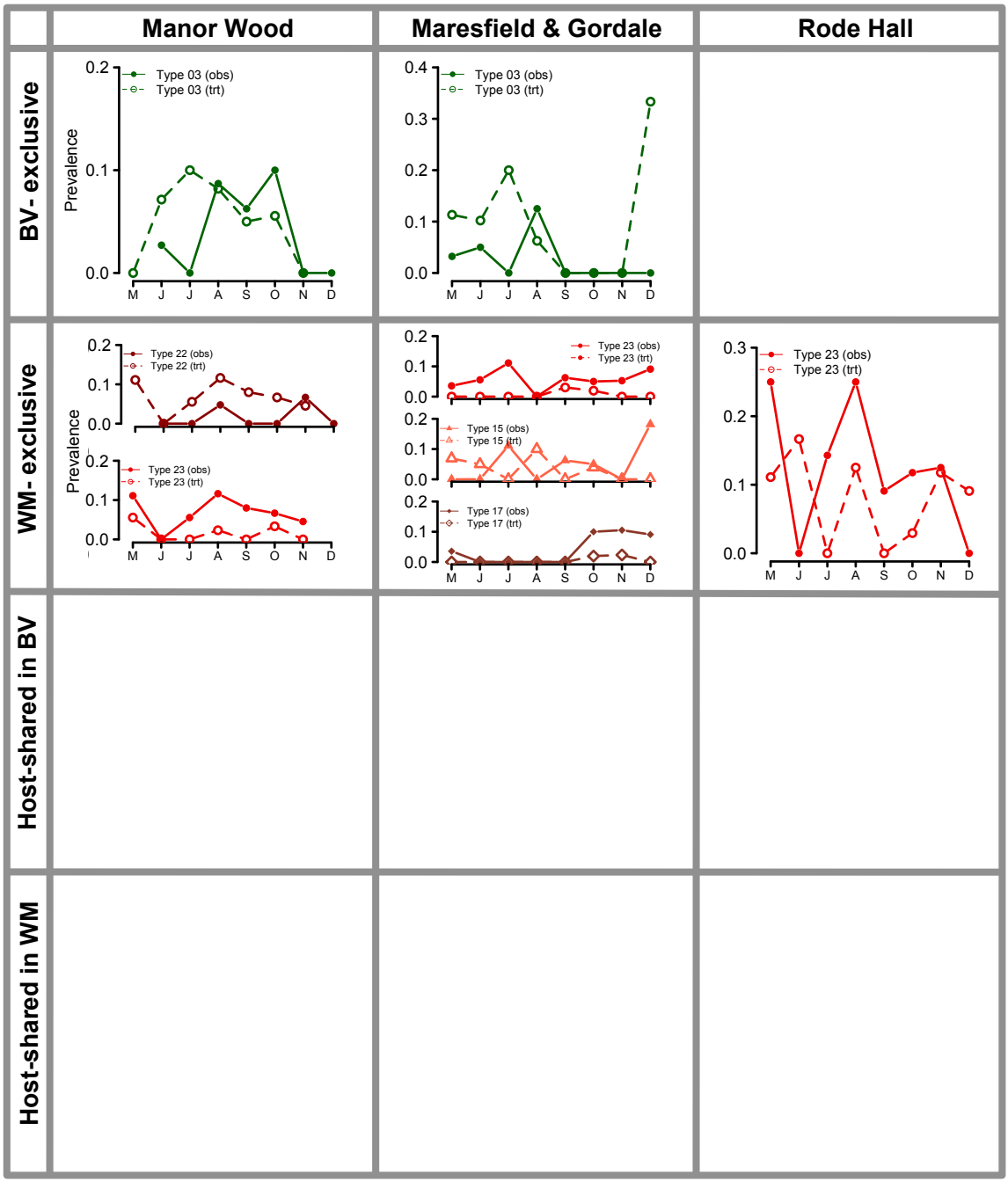

Figure 5.14: Seasonal prevalence of pITS variants of ***B. birtlesii*** in wood mice and bank voles on observation and treatment grids at the three woodland sites. Letters on x-axes represent months of the year from May to December. Variants are divided into those that are bank-vole exclusive, wood mouse-exclusive and host-shared. Plots in green relate to prevalence in bank voles; plots in red relate to prevalence in wood mice. Estimates of prevalence omit samples that were positive for infection but not sequenced. The prevalences here are therefore likely to be underestimates.

5.3.4.1 Models of infection risk with host-exclusive pITS variants

Bank vole-exclusive variants in bank voles

Sex was identified as a significant contributor to risk of infection with host-exclusive variants in bank voles and this factor was included in all subsequent models (χ^2=7.71, df=1, p=0.006). Models that included a negative effect of either of the cumulative number or proportion of bank voles previously treated were equally supported (Table 5.11; Figure 5.15). In both cases there was no support for an interaction with wood, indicating that the treatment effects were consistent across the three sites. The parameter estimates for all fixed effects within the best models of infection risk identified here (and for all other host-parasite combinations that follow) are given in Appendix 5.6.3.

Table 5.11: Model selection table for risk of infection in bank voles with bank vole-exclusive *Bartonella* variants. All models included a sinusoidal seasonal term, wood and individual sex as fixed effects. AICc for each model is given. Two models were equally supported (equally parsimonious and with lowest AICc within 2 units of each other) and are highlighted in yellow.

Model No.	Model	AICc
1.	Season + Wood	1111.6
2.	Season + Wood + Cumulative # treated	1108.7
3.	Season + Wood + Proportion of bank voles treated	1108.2
4.	Season + Wood + Proportion of rodent community treated	1110.3
5.	Season + Wood x Cumulative # treated	1112.1
6.	Season + Wood x Proportion of bank voles treated	1112.1
7.	Season + Wood x Proportion of rodent community treated	1113.7

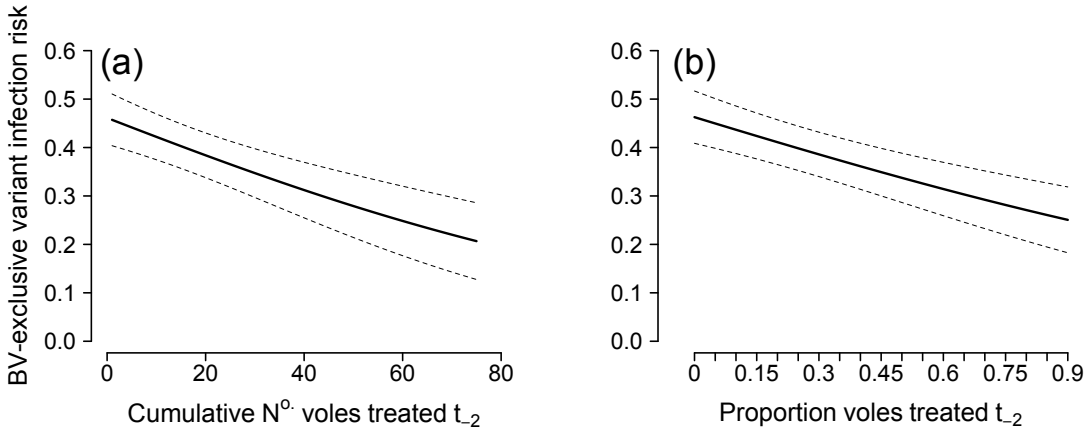

Figure 5.15: Predicted effect of Fipronil treatment of bank voles on infection risk with bank vole-exclusive *Bartonella* pITS variants in bank voles (dashed lines show standard errors). Two models that included different treatment effects were equally supported: (a) cumulative number of bank voles treated up to two months previously (b) proportion of bank voles treated two months previously. Both models indicated that increased treatment coverage caused a reduction in infection risk, and the effect was consistent across all three sites. The models also included a sinusoidal seasonality term and individual sex as fixed effects. These predictions relate to infection risk for males at MW in September.

Wood mouse-exclusive variants in wood mice

Individual age (χ^2=27.70, df=1, p<0.001) and an interaction between sex and reproductive condition (χ^2=9.14, df=1, p=0.001) were supported as contributors to infection risk in wood mice with host-exclusive *Bartonella* variants. These effects were included in all subsequent models of infection risk. There was no support for any treatment effects (Table 5.12), although one of the candidate models did not converge (model #2 in Table 5.12). However, the same model was identified as best when GLMs were used and AICc values were available for all models (Appendix 5.6.4).

Table 5.12: Model selection table for risk of infection in wood mice with wood mouse-exclusive *Bartonella* variants. All models included a sinusoidal seasonal term, wood, age and an interaction between sex and reproductive condition as fixed effects. AICc for each model is given. The best model (the most parsimonious model with lowest AICc) is highlighted in yellow.

Model No.	Model	AICc
1.	Season + Wood	1081.4
2.	Season + Wood + Cumulative # treated	DNC
3.	Season + Wood + Proportion of bank voles treated	1081.6
4.	Season + Wood + Proportion of rodent community treated	1081.8
5.	Season + Wood x Cumulative # treated	1082.6
6.	Season + Wood x Proportion of bank voles treated	1084.9
7.	Season + Wood x Proportion of rodent community treated	1085.0

5.3.4.2 Models of infection risk with host-shared pITS variants

The prevalence of shared variants in wood mice and bank voles at RH was particularly low (Table 5.10); therefore data from this site were not included in models of infection risk for either host species. In addition, models that included individual characteristics failed to converge, probably because of the relatively small number of samples in the data set that were positive for host-shared variants. As a result, no individual characteristics were included in any subsequent models.

Host-shared variants in bank voles

There was no support for any models that included treatment effects (Table 5.13), although two candidate models failed to converge (models #2 and #5 in Table 5.13). When models were run as GLMs without the random effect, there was support for a positive effect of the cumulative number of bank voles previously treated (Appendix 5.6.4; estimated coefficient = 0.03 ± 0.01[S.E.]), but this result should be treated with caution.

Host-shared variants in wood mice

There was no support for any models that included treatment effects (Table 5.13).

Table 5.13: Model selection table for risk of infection with host-shared *Bartonella* pITS variants in bank voles and wood mice. All models included a sinusoidal seasonal term and wood as fixed effects. Models did not include data from RH, as prevalence of host-shared *Bartonella* variants in both host species at this site was very low. AICc for each model is given. The best model (the most parsimonious model with lowest AICc) is highlighted in yellow. 'DNC' = model did not converge.

Model No.	Model	AICc	
		Bank Voles	Wood Mice
1.	Season + Wood	397.8	329.9
2.	Season + Wood + Cumulative # treated	DNC	331.7
3.	Season + Wood + Proportion of bank voles treated	396.7	331.9
4.	Season + Wood + Proportion of rodent community treated	397.1	331.9
5.	Season + Wood x Cumulative # treated	DNC	333.7
6.	Season + Wood x Proportion of bank voles treated	398.2	333.9
7.	Season + Wood x Proportion of rodent community treated	399.2	333.6

5.4 Discussion

Treatment of bank voles with Fipronil had a range of effects on the risk of *Bartonella* species infection in sympatric bank voles and wood mice, indicating that different transmission dynamics underlie the persistence of these parasites within this rodent community. In line with predictions, risk of wood mouse infection with the wood mouse-specific *B. doshiae*-like species was unaffected by treatment of bank voles, and while risk of *B. grahamii* infection (an apparently generalist parasite) in bank voles decreased as more of the bank vole population were treated, risk of infection in wood mice by the same species was unaffected. These results support the hypothesis that transmission between wood mice and bank voles is rare and is not responsible for persistence of these *Bartonella* species. However, contrary to predictions, risk of bank vole infection with the bank vole-specific *B. rudakovii* and with the apparently generalist *B. taylorii* and *B. birtlesii* was unaffected by treatment, and while risk of *B. taylorii* infection in wood mice was also unaffected, risk of *B. birtlesii* infection in wood mice decreased at two of the three sites. Overall, this suggests that the nature of transmission within this rodent system is subject to subtle complexities.

An absence of a reduced infection risk for some *Bartonella* parasites in bank voles could suggest that transmission between conspecifics is not necessary for persistence of *B. rudakovii*, *B. taylorii* or *B. birtlesii* within the bank vole population. Instead, transmission from another source may maintain infection risk. Wood mice are unlikely candidates for the source of *B. rudakovii* transmission, however, as my sampling of *Bartonella* infections never detected *B. rudakovii* infections in sympatric wood mice at these field sites (Chapter 3). Instead, field voles (*Microtus agrestis*), common shrews (*Sorex araneus*), or other unsampled sympatric species may be the key transmission hosts. Field voles and common shrews have been captured occasionally at these field sites (R. Barber, pers. comm.), and field voles are known to carry at least some of the same *Bartonella* spp. that were identified in wood mice and bank voles here (Telfer *et al*., 2007b), although it is unclear whether common shrews carry similar *Bartonella* species (Bray *et al*., 2007).

Wood mice are also an unlikely source of *B. taylorii* or *B. birtlesii* transmission to bank voles, as genetic characterisation of these apparently shared *Bartonella* species revealed that significantly different assemblages of *Bartonella* variants infected sympatric wood mice and bank voles at these sites (Chapter 3). However, some variants were found in both host species, and it is possible that continued transmission of host-shared variants from wood mice to bank voles maintained the risk of infection with these species in bank voles. In support of this notion, while treatment of the bank vole population saw a decrease in bank voles' risk of infection with bank vole-exclusive pITS variants, risk of infection with host-shared variants was unaffected in either host species. Transmission of shared variants appears to be driven by wood mice, and as such, I would expect to see a reduction in infection risk of host-shared variants in both host species following treatment of the wood mouse population. It should be noted, however, that models of infection risk for host-shared variants were problematic due to the relatively low prevalence of these infections, and confirmation of these patterns of treatment response would therefore benefit from an increased sample size. This could be achieved through more exhaustive sequencing of *Bartonella* infections, which may also increase the statistical power needed to seek effects of treatment for each pITS variant separately.

An alternative explanation for the fact that risk of infection with some *Bartonella* species was unaffected in bank voles following treatment is that the level of treatment was not great enough to significantly reduce the rate of transmission of these species within the bank vole population. Indeed, level of treatment coverage and compliance has been identified as a key determinant of population-wide success of applied control programmes (Boatin *et al*., 2012). It is also possible that the individual bank voles that were treated here were not those who contributed most to the force of infection of these *Bartonella* species. It is widely accepted that heterogeneities between individuals within a population result in a minority of the population being responsible for the majority of parasite transmission (i.e. the "20/80 rule"; Woolhouse *et al*., 1997). If these individuals were also less likely to be captured and treated, this may have resulted in a relatively strong force of infection remaining in the untreated bank vole population. Future experiments that aim to treat discrete demographic subsets of the bank vole population could provide further insight into this possibility.

As well as unpredicted responses to treatment on *Bartonella* spp. infection risk in bank voles, risk of *B. birtlesii* in wood mice was reduced following treatment of bank voles at Maresfield & Gordale, despite there being no obvious effect on risk of bank vole infection. This pattern may suggest that transmission of *B. birtlesii* within this rodent community is subject to a complex network of transmission, such that, using the terminology of Haydon *et al*. (2002), bank voles act as a source host for *B. birtlesii* infection in wood mice, but do not constitute a maintenance species in their own right. However, previous work (Chapter 3) has shown that the assemblages of *B. birtlesii* pITS variants that infect bank voles and wood mice are almost completely different at all three sites, including MFG. Furthermore, *B. birtlesii* infections were absent in bank voles at Rode Hall, but prevalence in wood mice at this site was high, indicating that transmission of *B. birtlesii* from bank voles to wood mice is not necessarily required for persistence of infection in wood mice. A reduction in wood mouse infection risk could arise if bank vole treatment reduced the overall size of the flea population available to infect wood mice. However, as the fleas in this system are both host generalists and generalist vectors of most *Bartonella* species (Chapter 4), I would expect this mechanism to elicit a concurrent reduction in the infection risk of other *Bartonella* species in wood mice and in bank voles, and this was not apparent. This reduction in wood mouse infection risk is therefore difficult to reconcile.

When modelled explicitly, risk of flea infection within the bank vole population was not reduced as a result of treatment, nor was an individual bank vole less likely to be infected with fleas if treated with Fipronil in the previous month compared to individuals who were not treated in the previous month. This indicates that Fipronil treatment did not prevent fleas from biting treated individuals, as predicted, and that the overall size of the pool of flea vectors was not affected. However, these results are in contrast to the findings of Smith *et al*. (2006), who studied the effects of Fipronil treatment on flea infection rates in populations of field voles (*Microtus agrestis*). They found a reduction in the prevalence of fleas in areas where field voles were treated with Fipronil compared to untreated control areas, and that individuals treated in the previous month were less likely to be infected with fleas. There are several possible reasons for the difference between the effect of Fipronil treatment on flea infection risk in the treated host species in the current study and the study by Smith *et al*. (2006). First, fleas that infect field voles may be more susceptible to the effects of Fipronil treatment

compared to those that infect bank voles. However, this seems unlikely, as several of the same flea species found to infect wood mice and bank voles here (Chapter 4) have been reported to infect field voles in the UK (Telfer *et al*., 2006). A second possibility is that absence of a treatment effect in the current study might result from the relatively short time period over which the study was conducted (six months) compared to the previous study. However, while Smith *et al*. (2006) found that risk of flea infection in areas exposed to treatment was lower in the second year of treatment compared to the first year, there was still an immediate reduction in infection risk, and this pattern was not found here. Finally, it is possible that abundant sympatric wood mice may have sustained the flea population in the current study, such that any losses to the flea population as a result of bank vole treatment were mitigated by continued, and possibly increased, reproduction of fleas that feed on wood mice. Assuming that bank voles, wood mice and fleas mix at random, risk of flea infection in bank voles would have been sustained. In contrast, field voles dominated the rodent communities studied by Smith *et al*. (75% of captures were field voles according to a different study at the same site; Telfer *et al*., 2007b), and it is therefore less likely that other host species would have been able to maintain the flea population to a similar size in the presence of field-vole-targeted treatment.

Given the absence of an effect of treatment on risk of flea infection in bank voles, it is surprising that treatment appeared to affect risk of flea infection in wood mice. Risk of infection decreased in Manor Wood with an increase in the proportion of the bank vole population, or the total rodent community, that were treated two months previously. Bank voles dominated the rodent communities at Manor Wood for much of the sampling period, so this host species may be key to maintaining the flea population at this site. However, without a concurrent decrease in bank vole infection risk as a response to treatment, reduced infection risk in wood mice following treatment is difficult to explain. Furthermore, treatment had the opposite effect in Maresfield & Gordale: infection risk in wood mice increased with an increase in treatment coverage two months previously. It should be noted that infection risk on treatment grids at Maresfield & Gordale was consistently higher than on the control grid, even before the onset of bank vole treatment. This apparent increase in risk following treatment may therefore be an artefact of underlying differences between treatment and control grids at this site. It should also be noted that the most common flea species identified within

these study sites (*C. n. vulgaris*; Chapter 4) has been classified as a "nest flea" (spending a large proportion of its life within the host's nest, and only remaining on-host for the duration of feeding; Marshall, 1981; Krasnov, 2008). It is therefore likely that sampling of fleas directly from trapped hosts (including wood mice and bank voles) and subsequent estimation of flea prevalence may be unreliable. Greater insight into the size of the flea population associated with each host species, and responses to Fipronil treatment, may be gleaned by more direct sampling of fleas within the hosts' nests.

Attempts to manipulate the natural flea-mediated transmission dynamics of *Bartonella* between wood mice and bank voles in their natural environments have provided new insight into the complexities of parasite persistence within this rodent community. In many cases, this experiment confirmed the results of previous genetic characterisation of *Bartonella* species (Chapter 3) that suggested transmission of *Bartonella* between wood mice and bank voles is rare, and experiments in which wood mice are targeted with treatment would be useful for further confirmation of this. More generally, these results highlight the fact that broadly sympatric distributions of host species do not necessarily predict the occurrence of between-species transmission, and that more fine-scale characterisations of host species interactions, both spatially and temporally, are likely to be more useful for predicting the occurrence of parasite transmission between them.

This experiment also highlighted further complexities to transmission within this system that deserve further consideration. Firstly, other host species besides wood mice and bank voles may be implicated in the persistence of some *Bartonella* parasites and this possibility should be investigated. As other species were apparently very rare at these field sites, this emphasizes the fact that key host species are not necessarily those that are most abundant within a community (Kilpatrick *et al*., 2006; Streicker *et al*., 2013). Finally, the persistence of vector-borne parasites depends on both the size of the vector population and the prevalence of infection within the vector population, which may be affected in different ways by different host species within a community (e.g. Gilbert *et al*., 2001). Here, the results of targeted vector-removal on parasite prevalence within wood mice and bank voles were often difficult to reconcile given the predicted mechanisms of the treatment, and key host species could not be determined. This may result from one or both host species affecting the size of the flea population as well as

the rate of flea infection with *Bartonella*. Work is now needed to tease apart the exact influences (i.e. flea population size and/or flea infection rate) that each host species has on the force of *Bartonella* species infection in the community.

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5.6 Appendix

5.6.1 Coefficient estimates for all best models of flea and *Bartonella* spp. infection risk, according to GLMMs.

Table A5.1: Coefficient estimates and standard errors for the fixed effects in the best models of flea infection risk. There were two equally supported models for infection risk in wood mice, therefore coefficient estimates for both models are given.

		β	S.E.	*z*	*p*
Bank Voles					
	Intercept	-2.826	0.336	-8.411	<0.001
	Reproductive condition: not active	0.434	0.215	2.022	0.04
	Cos12	0.262	0.158	1.660	0.10
	Sin12	1.030	0.292	3.526	<0.001
	Wood: RH	0.127	0.212	0.600	0.55
	Wood: MFG	0.087	0.218	0.399	0.69
Wood Mice					
	Intercept	-2.724	0.306	-8.910	<0.001
	Cos12	0.359	0.217	1.650	0.10
	Sin12	0.833	0.286	2.913	0.004
	Prop. BV. trt. t-2	-2.654	1.698	-1.563	0.12
	Wood: RH	-0.551	0.394	-1.399	0.16
	Wood: MFG	0.034	0.292	0.117	0.91
	Prop.BV.trt.t-2 x Wood: RH	3.058	1.852	1.651	0.10
	Prop.BV.trt.t-2 x Wood: MFG	3.710	1.706	2.175	0.03
	Intercept	-2.678	0.297	-9.010	<0.001
	Cos12	0.375	0.218	1.717	0.09
	Sin12	0.759	0.275	2.760	0.006
	Prop.comm.trt. t-2	-3.851	2.570	-1.498	0.13
	Wood: RH	-0.602	0.399	-1.511	0.13
	Wood: MFG	0.053	0.292	0.183	0.854
	Prop.comm.trt.t-2 x Wood: RH	4.652	2.732	1.703	0.09
	Prop.comm.trt.t-2 x Wood: MFG	5.718	2.642	2.164	0.03

Table A5.2: Coefficient estimates and standard errors for the fixed effects in the best models of *B. grahamii* infection risk. There were two equally supported models for infection risk in bank voles, therefore coefficient estimates for both models are given.

		β	S.E.	*z*	*p*
Bank Voles					
	Intercept	-1.817	0.328	-5.542	<0.001
	Age: Young	0.514	0.246	2.089	0.04
	Treated last session: Yes	-0.719	0.512	-1.404	0.160
	Sin12	-0.870	0.215	-4.040	<0.001
	Cos12	0.125	0.306	0.409	0.68
	Cumulative.#.trt.t-2	-0.021	0.009	-2.270	0.02
	Wood: RH	-0.947	0.286	-3.314	<0.001
	Wood: MFG	-0.819	0.306	-2.674	0.007
	Intercept	-1.881	0.319	-5.893	<0.001
	Age: Young	0.521	0.245	2.128	0.03
	Treated last session: Yes	-0.580	0.516	-1.124	0.26
	Sin12	-0.942	0.224	-4.206	<0.001
	Cos12	0.175	0.292	0.601	0.55
	Prop.BV.trt.t-2	-1.531	0.605	-2.531	0.01
	Wood: RH	-0.861	0.284	-3.034	0.002
	Wood: MFG	-0.683	0.305	-2.242	0.02
Wood Mice					
	Intercept	-9.919	1.001	-9.830	<0.001
	Sin12	-1.394	0.442	-3.153	0.002
	Cos12	1.918	0.534	3.592	<0.001
	Wood: MFG	0.373	0.794	0.470	0.64

Table A5.3: Coefficient estimates and standard errors for the fixed effects in the best models of *B. taylorii* infection risk.

		β	S.E.	*z*	*p*
Bank Voles					
	Intercept	-3.191	0.427	-7.469	<0.001
	Sin12	0.152	0.222	0.687	0.492
	Cos12	0.788	0.331	2.382	0.017
	Wood: RH	1.665	0.293	5.689	<0.001
Wood Mice					
	Intercept	-1.222	0.281	-4.356	<0.001
	Age: Young	-0.910	0.276	-3.296	<0.001
	Sex: Male	0.787	0.279	2.818	0.005
	Reproductive condition: not active	0.219	0.257	0.852	0.29
	Sin12	-0.063	0.133	-0.472	0.637
	Cos12	0.207	0.158	1.310	0.190
	Wood: RH	0.082	0.221	0.370	0.711
	Wood: MFG	0.110	0.200	0.554	0.580
	Sex: Male, Repro. condition: not active	-0.770	0.334	-2.309	0.021

Table A5.4: Coefficient estimates and standard errors for the fixed effects in the best models of *B. birtlesii* infection risk.

		β	S.E.	*z*	*p*
Bank Voles					
	Intercept	-10.02	1.008	-9.944	<0.001
	Sin12	-1.713	0.479	-3.574	<0.001
	Cos12	2.001	0.677	2.958	0.003
	Wood: MFG	0.505	0.800	0.632	0.53
Wood Mice					
	Intercept	-2.193	0.329	-6.666	<0.001
	Age: Young	-0.646	0.339	-1.905	0.06
	Sin12	-0.055	0.165	-0.332	0.74
	Cos12	-0.274	0.196	-1.403	0.16
	Cumulative # BV trt t-2	-0.005	0.008	-0.674	0.500
	Wood: RH	1.602	0.358	4.474	<0.001
	Wood: MFG	1.104	0.316	3.496	<0.001
	Cum.#.trt.t-2: WoodRH	-0.010	0.011	-0.932	0.35
	Cum.#.trt.t-2: WoodMFG	-0.037	0.016	-2.317	0.020

Table A5.5: Coefficient estimates and standard errors for the fixed effects in the best models of *B. rudakovii* infection risk.

		β	S.E.	*z*	*p*
Bank Voles					
	Intercept	-9.163	0.849	-10.79	<0.001
	Sin12	-1.256	0.384	-3.269	0.001
	Cos12	-0.237	0.444	-0.535	0.59
	Wood: RH	0.934	0.818	1.141	0.25
	Wood: MFG	1.007	0.871	1.157	0.25

Table A5.6: Coefficient estimates and standard errors for the fixed effects in the best models of *B. doshiae*-like infection risk.

		β	S.E.	*z*	*p*
Wood Mice					
	Intercept	-8.393	0.867	-9.679	<0.001
	Age: Young	-3.858	1.982	-1.946	0.05
	Sin12	-0.053	0.382	-0.138	0.89
	Cos12	-0.422	0.382	-1.106	0.269
	Wood: RH	-0.423	0.963	-0.439	0.660
	Wood: MFG	-0.823	0.903	-0.911	0.362

5.6.2 GLM model selection tables for models of flea and *Bartonella* spp. infection risk in wood mice and bank voles.

All models include the same fixed effects as when run as GLMMs.
All of the same best models were identified as with the GLMM analysis.

Table A5.7: Flea infection risk.

Model	Bank Voles	Wood Mice
Season + Wood	904.6	609.0
Season + Wood + C # trt t_{-2}	906.5	610.8
Season + Wood + Prop. voles. trt t_{-2}	906.6	609.9
Season + Wood + Prop. comm. trt t_{-2}	906.6	610.4
Season + Wood x C. # trt t_{-2}	904.3	607.5
Season + Wood x Prop.voles. trt t_{-2}	906.5	605.4
Season + Wood x Prop. comm. trt t_{-2}	907.6	606.0

Table A5.8: *B. grahamii* infection risk.

Model	Bank Voles	Wood Mice
Season + Wood	854.6	554.7
Season + Wood + C # trt t_{-2}	850.8	553.0
Season + Wood + Prop. voles. trt t_{-2}	849.3	554.9
Season + Wood + Prop. comm. trt t_{-2}	851.8	555.5
Season + Wood x C. # trt t_{-2}	853.4	554.4
Season + Wood x Prop.voles. trt t_{-2}	853.1	556.8
Season + Wood x Prop. comm. trt t_{-2}	854.8	557.4

Table A5.9: *B. taylorii* infection risk.

Model	Bank Voles	Wood Mice
Season + Wood	710.8	1441.5
Season + Wood + C # trt t_{-2}	710.0	1440.6
Season + Wood + Prop. voles. trt t_{-2}	710.8	1442.8
Season + Wood + Prop. comm. trt t_{-2}	711.7	1442.6
Season + Wood x C. # trt t_{-2}	711.6	1443.7
Season + Wood x Prop.voles. trt t_{-2}	712.5	1445.7
Season + Wood x Prop. comm. trt t_{-2}	713.0	1444.3

Table A5.10: *B. birtlesii* infection risk.

Model	Bank Voles	Wood Mice
Season + Wood	725.7	1194.4
Season + Wood + C # trt t_{-2}	725.8	1192.7
Season + Wood + Prop. voles. trt t_{-2}	725.5	1192.9
Season + Wood + Prop. comm. trt t_{-2}	726.5	1195.0
Season + Wood x C. # trt t_{-2}	726.9	1191.2
Season + Wood x Prop.voles. trt t_{-2}	726.8	1196.8
Season + Wood x Prop. comm. trt t_{-2}	727.3	1198.5

Table A5.11: *B. doshiae*-like infection risk.

Model	Wood Mice
Season + Wood	824.6
Season + Wood + C # trt t_{-2}	822.8
Season + Wood + Prop. voles. trt t_{-2}	823.1
Season + Wood + Prop. comm. trt t_{-2}	823.3
Season + Wood x C. # trt t_{-2}	826.2
Season + Wood x Prop.voles. trt t_{-2}	825.4
Season + Wood x Prop. comm. trt t_{-2}	825.6

Table A5.12: *B. rudakovii* infection risk.

Model	Bank Voles
Season + Wood	814.3
Season + Wood + C # trt t_{-2}	815.8
Season + Wood + Prop. voles. trt t_{-2}	816.2
Season + Wood + Prop. comm. trt t_{-2}	816.2
Season + Wood x C. # trt t_{-2}	819.8
Season + Wood x Prop.voles. trt t_{-2}	819.8
Season + Wood x Prop. comm. trt t_{-2}	919.9

5.6.3 Coefficient estimates for fixed effects in the best models of variant-level infection risk

Table A5.13: Coefficient estimates and standard errors for the fixed effects in the best models of infection risk for bank vole-exclusive variants in bank voles. Two models were equally supported.

		β	S.E.	*z*	*p*
Bank Vole exclusive variants in BV					
Model 1					
	Intercept	-1.115	0.252	-4.422	<0.001
	Sex: Male	0.336	0.175	1.914	0.06
	Sin12	-0.578	0.161	-3.591	<0.001
	Cos12	0.214	0.241	0.886	0.38
	Cumulative#trt.t-2	-0.016	0.007	-2.161	0.03
	Wood:RH	0.675	0.205	3.298	<0.001
	Wood: MFG	-0.564	0.231	-2.446	0.01
Model 2					
	Intercept	-1.185	0.239	-4.948	<0.001
	Sex: Male	0.352	0.176	2.002	0.05
	Sin12	-0.642	0.172	-3.738	<0.001
	Cos12	0.254	0.231	1.103	0.27
	Prop.BV.trt.t-2	-1.051	0.458	-2.295	0.02
	Wood:RH	0.748	0.209	3.600	<0.001
	Wood: MFG	-0.447	0.233	-1.919	0.06s

Table A5.14: Coefficient estimates and standard errors for the fixed effects in the best models of infection risk for wood mouse-exclusive variants in wood mice.

		β	S.E.	*z*	*p*
WM-exclusive variants in WM					
	Intercept	-0.846	0.327	-2.588	0.01
	Age: Young	-1.200	0.326	-3.682	<0.001
	Sex: Male	1.101	0.358	3.078	0.002
	Reproductive condition: Not active	-0.084	0.305	-0.276	0.78
	Sin12	0.129	0.166	0.779	0.44
	Cos12	0.185	0.194	0.955	0.34
	Wood:RH	1.034	0.286	3.617	<0.001
	Wood:MFG	0.641	0.249	2.573	0.01
	SexMale: Reprodictive condition: not	-1.213	0.422	-2.874	0.004s

Table A5.15: Coefficient estimates and standard errors for the fixed effects in the best models of infection risk for host-shared variants in bank voles and wood mice.

		β	S.E.	*z*	*p*
Shared variants in BV					
	Intercept	-2.132	0.359	-5.936	<0.001
	Sin12	0.694	0.253	2.738	0.006
	Cos12	0.124	0.426	0.292	0.771
	Wood: MFG	-0.939	0.336	-2.795	0.005
Shared variants in WM					
	Intercept	-10.440	1.213	-8.608	<0.001
	Sin12	-1.743	0.622	-2.803	0.005
	Cos12	1.145	0.659	1.737	0.08
	Wood: MFG	0.349	1.001	0.349	0.727

5.6.4 GLM model selection tables for models of *Bartonella* variant (host-exclusive and host-shared) infection risk in wood mice and bank voles.

All models include the same fixed effects as when run as GLMMs.

Table A5.16: Bank vole-exclusive variants in bank voles.

Model	Bank Voles
Season + Wood	1117.0
Season + Wood + C # trt t_{-2}	1114.4
Season + Wood + Prop. voles. trt t_{-2}	1114.0
Season + Wood + Prop. comm. trt t_{-2}	1116.0
Season + Wood x C. # trt t_{-2}	1118.0
Season + Wood x Prop.voles. trt t_{-2}	1117.8
Season + Wood x Prop. comm. trt t_{-2}	1119.4

Table A5.17: Wood mouse-exclusive variants in wood mice.

Model	Wood Mice
Season + Wood	1099.2
Season + Wood + C # trt t_{-2}	1097.7
Season + Wood + Prop. voles. trt t_{-2}	1099.5
Season + Wood + Prop. comm. trt t_{-2}	1099.6
Season + Wood x C. # trt t_{-2}	1101.6
Season + Wood x Prop.voles. trt t_{-2}	1103.0
Season + Wood x Prop. comm. trt t_{-2}	1102.4

Table A5.18: Host-shared variants in bank voles and wood mice.

Model	Bank Voles	Wood Mice
Season + Wood	395.8	403.4
Season + Wood + C # trt t_{-2}	390.3	403.7
Season + Wood + Prop. voles. trt t_{-2}	394.7	403.4
Season + Wood + Prop. comm. trt t_{-2}	395.1	404.1
Season + Wood x C. # trt t_{-2}	392.0	404.0
Season + Wood x Prop.voles. trt t_{-2}	396.1	405.4
Season + Wood x Prop. comm. trt t_{-2}	397.1	406.1

Chapter 6
General Discussion

Infectious diseases continue to threaten global health and biodiversity, and have serious economic impacts through the costs of treatment regimes and the loss of livestock production (Daszak *et al*., 2000; Morens *et al*., 2004). As most parasites in nature exist within multi-host communities, a sound understanding of how different species contribute to parasite persistence is vital for the design of effective control measures. The work presented here provides novel insights into the complex nature of parasite transmission within an amenable host community that are also of relevance for the study of multi-host parasite transmission dynamics in general. In particular, it raises questions about the existence of true multi-host parasites that ought to be considered in future studies of parasite transmission and control within communities of multiple host species.

Overview of findings

Focusing on a model multi-host-multi-parasite system within its natural environment, the work presented in this thesis sought to understand the transmission dynamics responsible for the persistence of several endemic *Bartonella* parasites within sympatric populations of wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*). In line with previous studies (Birtles *et al*., 2001; Telfer *et al*., 2007a), I found that *Bartonella* infections were common in wild rodent communities at my study sites in northwest England, and found several different species circulating within sympatric wood mouse and bank vole populations (Chapter 2). Furthermore, and also corroborating these previous studies, three species of *Bartonella* were found to infect both host species (*B. grahamii*, *B. taylorii* and *B. birtlesii*). However, one of the most notable findings of this work was that single species of *Bartonella* often comprised a complex of genetic variants, as revealed by sequencing at a partial fragment of the 16S-23S rDNA intergeneic spacer region (pITS) (Chapter 3). Furthermore, genetic characterisation of *Bartonella* parasites that appeared to be generalists at the species level revealed that different variants were often closely associated with different host species, suggesting that transmission between species was actually rare. This notion was supported by experimental manipulation of between-species transmission within these

wild communities, and was best identified by grouping the parasites on a 'functional' (i.e. host-exclusive versus host-shared variants) rather than taxonomic (i.e. *Bartonella* species) basis. Overall, these findings suggest that apparently generalist parasite species may, in fact, be relatively host-specific when examined in closer detail.

Potential barriers to cross-species transmission

In Chapter 4, I investigated whether a lack of *Bartonella* transmission between wood mice and bank voles was the result of either barriers to encounter or incompatibility between host species and *Bartonella* variants (Combes, 2001). In particular, I sought to determine whether there was evidence for current encounter barriers mediated by specific associations between rodent hosts and the flea species that vector the *Bartonella* parasites, by identifying the flea species present on individual wood mice and bank voles, and characterising the *Bartonella* parasites that they were carrying. In line with the findings of others (e.g. Telfer *et al*., 2005; Noyes *et al*., 2002) I found little evidence in support of specific host-flea associations. Broadly the same flea species were found to infest wood mice and bank voles, and there was evidence to suggest that at least two flea species (*Ctenophthalmus nobilis vulgaris* and *Megabothris turbidus*) were able to transfer between individuals of different host species (based on the identity of *Bartonella* variants they were carrying; Chapter 4). Overall, these results suggest that any host-exclusivity among *Bartonella* variants, and lack of between-species transmission, is therefore more likely to arise through different compatibility between host species and *Bartonella* variants, rather than as a result of current ecological encounter barriers (e.g. through differential *Bartonella*-flea or flea-rodent specificity).

Genetic variation and host-specificity of **Bartonella** *infections in rodent communities*

The existence of sub-species genetic variation of *Bartonella* parasites, coupled with the underlying variant-host specificity of apparently host-generalist *Bartonella* species identified in Chapter 3, puts into question the value of previous studies of *Bartonella* in woodland rodents in the UK that have not accounted for this genetic diversity. For example, in Chapter 2, I attempted to determine the relative contributions of wood mice and bank voles to community force of infection using species-level identifications of *Bartonella* infections. The identity of a key transmission host was largely unresolved

for all *Bartonella* species, and this is likely to be because transmission between host species is rare and limited to a few host-shared variants (although the role of host density effects on the population dynamics of the flea vectors cannot be dismissed as a complicating factor here). The study by Telfer *et al*. (2007a) also used species-level identifications of infections in wood mice and bank voles to investigate whether the coexistence of multiple *Bartonella* species within the same woodland rodent communities is the result of differences in seasonality and/or host preferences. They compared the seasonal dynamics of *Bartonella* species, their dependencies on host density, and prevalences within wood mice and bank voles, and concluded that several species differed in these ecological traits. However, the results presented in this thesis indicate that genetic variants of the same *Bartonella* species vary considerably in their host species associations. Investigations of *Bartonella* ecology at the species level therefore conceal the ecological differences between variants of the same species, which may obscure any conclusions relating to the mechanisms of parasite coexistence and the transmission dynamics that facilitate their persistence within these communities. Future studies should therefore endeavour to identify *Bartonella* infections to sub-species level.

Indeed, sub-species genetic variation of *Bartonella* parasites coupled with specific host associations have been demonstrated elsewhere in other rodent communities. For example, experimental infections of rodents in the USA found that bacteremia in cotton rats (*Sigmodon hispidus*) and white-footed mice (*Peromyscus leucopus*) would only establish when hosts were inoculated with the same citrate synthase (*glt*A) *Bartonella* variant that was originally obtained from the same species (Kosoy *et al*., 2000). Also, in a study of rodent *Bartonella* infections in Poland, several *glt*A variants were detected, and significant associations were found between clades of *Bartonella* variants and host species according to nested clade analysis (Paziewska *et al*., 2011). The results found here, which used a different genetic marker, therefore reinforce the notion that between-species transmission of *Bartonella* may be relatively uncommon in the wild, and that dynamics and persistence of infection within each host species is unlikely to depend on cross-species transmission.

Re-evaluating diagnostic methods for rodent Bartonella *infections*

In recognising the high degree of genetic diversity within individual *Bartonella* species, the work here highlights limitations to the method used for the species-level identification of *Bartonella* infections in this thesis and other previous studies (e.g. Telfer *et al*., 2005, 2007a, 2007b). This method was based on length polymorphism of a partial fragment of the 16S-23S rDNA intergenic spacer region (ITS) (Roux & Raoult, 1995; Birtles *et al*., 2000; Houpikian & Raoult, 2001; Telfer *et al*., 2005). This is a valuable high-throughput technique that enables rapid diagnosis of *Bartonella* infections directly from field-collected blood samples. However, as demonstrated here, this method conceals the high genetic variability within *Bartonella* species, and importantly, fine-scale associations between sub-specific variants and different host species. In addition, my sequencing analysis also confirmed that several distinct *Bartonella* species cannot be distinguished based on ITS length polymorphism, and as a result, there may be inconsistencies with previous reporting of some rodent *Bartonella* infections. For example, BGA and *B. rudakovii* have a similar pITS length. An earlier study reported BGA infections in bank voles at Manor Wood (Telfer *et al*., 2007a), but through my sequencing analysis (see Chapter 3) I found that bank voles were never infected with BGA, but were infected with *B. rudakovii* (Knap *et al*., 2007). Similarly, the pITS lengths of *B. doshiae* and an unnamed species referred to as '*B. doshiae*-like' (Telfer *et al*., 2005; Birtles *et al*., 2001) are similar. *B. doshiae* infections were previously reported in wood mice (Telfer *et al*., 2007a), but my sequencing analysis identified all infections of that pITS length in wood mice were '*B. doshiae*-like'. Future studies that investigate the nature of *Bartonella* transmission in multi-host rodent communities should therefore use finer-scale diagnostic methods in order to fully appreciate the identity and sub-species variation of these parasites, and their potentially different underlying transmission dynamics.

Using experiments to understand parasite transmission dynamics

Fine-scale identification of parasite infections will facilitate studies of parasite transmission dynamics within multi-host communities. However, analyses and interpretation of observational data from natural systems are always likely to be challenging, due to the inherent limitations of such data (e.g. see Smart *et al*., 2012). For example, environmental variation often creates noisy data, which makes it difficult

to identify patterns that are caused by the biological processes of interest (e.g. annual variation in temperatures may affect the population dynamics of flea vectors within the rodent-*Bartonella* system, confounding assessments of host contributions to infection risk). Furthermore, with no control over the variation of potential explanatory variables, collinearity between variables is likely, which can cause problems with parameter estimation and model selection. However, in Chapter 5 I demonstrated that experimental manipulation of *Bartonella* transmission within natural rodent communities is one potentially useful strategy for teasing apart meaningful biological processes from environmental variation.

Such experimental approaches are being increasingly recognised as a useful tool for studying parasite transmission, and interactions between parasite species, in natural systems. For example, Smith *et al*. (2006) treated natural populations of field voles (*Microtus agrestis*) with Fipronil to remove their fleas to investigate the mode of *Trypanasoma microti* transmission within this host species. They found that while prevalence of flea infection in field voles decreased substantially following treatment, prevalence of flea-borne *Trypanasoma microti* was not reduced to as great a degree, indicating a potential role for direct transmission of this parasite. In another example, Knowles *et al*. (2013) treated individual wild wood mice with anthelmintics to remove intestinal helminths and to assess the presence and strength of interactions between coinfecting parasites. They found evidence of local, dynamic competition between intestinal helminths and *Eimeria* protozoa, as anthelmintic treatment resulted in strong, but short-lived, increases in *Eimeria* abundance. Similar experiments have also been conducted at a much larger scale: the immune-mediated interactions between coinfecting gastrointestinal helminths and bacteria causing bovine tuberculosis in free-ranging African buffalo have been studied using experimental anthemlinthic treatments (Ezenwa *et al*., 2010). While such experiments within natural systems are ambitious and not without their limitations, they do offer invaluable insight into parasite interactions and transmission dynamics that bridge the gap between the unnatural but controlled conditions of laboratory experiments and the quasi-experiments afforded by applied disease control interventions.

Genetic variation and host specificity of parasites within multi-host communities

It is likely that 'covert' host specificity of apparently generalist parasites is a widespread phenomenon, and deserves further consideration in the study of endemic parasite infection dynamics within multihost communities. Several studies have shown that genetic diversity of parasite populations in often high (Poulin & Keeney, 2008), and that parasite species found to be host-generalists when initially identified (e.g. by morphological characteristics, or by coarse genetic characterisation) are found to comprise several distinct host-specific species or strains when characterised at a more fine-scale genetic level (e.g. Donald *et al*., 2004; Sehgal *et al*., 2005; Whiteman *et al*., 2006). It therefore follows that identifying parasitic infections at as fine a scale as possible is important in order to gain a clear understanding of the structure of the parasite "maintenance community" (Haydon *et al*., 2002), and to plan control interventions appropriately.

The practical importance of identifying the genetic structure of parasite populations has been demonstrated for the transmission of the parasitic nematode *Schistosoma japonicum* in diverse host communities in China. Wang *et al*. (2006) characterised the population structure of *S. japonicum* miracidia expelled by seven different host species, and found that parasites infecting humans and bovids were more similar to each other than either were to parasites infecting goats, pigs, dogs or cats. This suggested that transmission between humans and bovines was more common than between humans and other domestic animals, and furthermore, that transmission between bovines and other domestic animals was rare. Recent theoretical work has further demonstrated that bovines are both the maintenance and source species for transmission of *S. japonicum* to humans, being able to maintain infection in the absence of transmission from other host species. A reduction in human infection risk would therefore require removal of this host species alone (Rudge *et al*., 2013).

The evolutionary stability of host-parasite specificity

The widespread host-specificity of parasites that exist within multi-host communities begs the question of how such specificity arises, and whether it is stable over time. Experimental infections in a broad range of systems have demonstrated that parasites

are often only able to cause infection in a limited number of host species when given the opportunity (Tompkins & Clayton, 1999; Kosoy *et al*., 2000; Komar *et al*., 2003; Esberard *et al*., 2005; Gilbert & Webb, 2007; Palinauskas *et al*., 2008). Through studying the relationships between host species, flea species and *Bartonella* variants here, I also concluded that the opportunity for different *Bartonella* variants to infect both host species was evident, but that many variants infected only one species. Such findings indicate that host-parasite incompatibility plays a greater role in determining host-specificity than current ecological barriers to host-parasite encounter.

However, current associations between hosts and parasites, including those identified between rodents and *Bartonella* variants in this study, represent a snapshot in evolutionary time, which do not betray the evolutionary pressures that led to them. Understanding how contemporary and historical selective pressures shape current host-parasite associations (Vander Wal *et al*., 2014) may be informative with respect to the stability of such associations, indicating how readily they may break down under different ecological or environmental conditions. For example, current incompatibility between a host and parasite species/variant may have arisen due to local adaptation to a different host species as a result of previous ecological barriers to encounter. Restricted transmission of parasites within isolated groups of individuals reduces gene flow and may generate genetic structure within a parasite population (Bruyndonckx *et al*., 2009; Ruiz-Gonzalez *et al*., 2012). As parasites are often more numerous and have shorter generation times than their hosts, genetic bottlenecks may give rise local adaptation of parasites, where a parasite is only able to exploit a single host species even if the opportunity to exploit other hosts arises (Yourth & Schmid-Hempel, 2006; Little *et al*., 2006).

If such mechanisms do underpin patterns of host-parasite compatibility, including those between rodents and *Bartonella* variants demonstrated in this thesis, it suggests that the very nature of this compatibility may be transient, and subject to the dynamic interactions of hosts and parasites (and/or vectors) within the community, which may change over time (Ruiz-Gonzalez *et al*., 2012). Depending on the relative current fitness benefits of a host-specific versus host-generalist parasite strategy, it may be that apparently host-specific parasites are in the process of evolving to become generalists (through repeated encounters with new host species that facilitate host-switching; Antia

et al., 2003, Lloyd-Smith *et al*., 2009) or that current generalists are evolving to become specialists (through newly imposed structure of host populations that cause bottlenecks within the parasite population). The challenge is identifying where on the evolutionary continuum a parasite is currently placed, and how this might infringe on the success of control interventions that are likely to be designed without evolutionary processes borne in mind. An advantage of natural model systems, like the rodent-*Bartonella* system studied here, is the relative ease with which populations of host species and the genetic structure of their associated parasite populations can be monitored through time. For example, a comparison of host-*Bartonella* associations found here with those found during previous studies of rodent *Bartonella* infection dynamics in multi-host communities (e.g. Telfer *et al*., 2007a), if possible, would provide valuable insight into the nature of the selective pressures driving the evolution of specificity or generalism.

Do 'true multi-host parasites' exist?

The community context of host-parasite interactions is justifiably a growing area of research within parasite ecology (Fenton & Pedersen, 2005; Pedersen & Fenton, 2006; Gortazar *et al*., 2007; Telfer *et al*., 2010), and the fact that "most parasites are able to infect multiple host species" is now a common and readily accepted phrase. However, the work I have presented here supplements a growing body of evidence that parasites that circulate endemically within multi-host communities are generally more host-specific than they initially appear. The ability to infect multiple host species is undoubtedly an important consideration in determining the likelihood of a parasite species to cause spillover infection in different host species, and a major risk factor in the emergence of a parasite into novel host species (Taylor *et al*., 2001; Cleaveland *et al*., 2001). However, it seems that once a host-jump has been made and parasite establishment within a new host species has been successful, the evolution of host-specific strains that are incapable of infecting other host species even when presented with the opportunity may be commonplace (e.g. Donald *et al*., 2004; Sehgal *et al*., 2005; Whiteman *et al*., 2006). As such, a 'true multi-host parasite' (one that experiences high rates of both within- and between-species transmission; Fenton & Pedersen, 2005) may rarely exist in nature.

With the advent of evermore-sophisticated genomics technology, the fine-scale characterisation of parasite infections is becoming increasingly available and will provide valuable insight into the ecology of parasites that circulate endemically within multi-host communities (Forrester & Hall, 2014). I believe it is therefore time to debate the existence of the 'true multi-host parasite'; is transmission from one host species to another ever important in endemic disease persistence in multi-host communities and, if not, how should this affect future approaches to disease control? The need for such a debate may simply be rooted in semantics; it has long been recognised that parasites often produce many different genetic variants, some of which become associated with different host species (e.g. rabies virus; Rupprecht *et al*., 1987), and many authors continue to refer to such parasites as multi-host parasites (e.g. Woolhouse *et al*., 2001). However, if the genetic structure of a parasite population is such that different strains routinely infect different host species, is it appropriate to refer to that species of parasite as a 'multi-host' parasite? I would argue that, regardless of its evolutionary history, if, on an ecological time-scale, the endemic persistence of a parasite within a population of a particular host species does not depend on transmission from or to a different host species, its classification as a multi-host parasite, 'true' or otherwise, is not justified.

In many ways, this may appear to simplify any approaches to endemic disease control within multi-host communities, as such patterns of host-specificity suggest that control interventions need to be targeted at reducing transmission only within populations of the host species of concern. However, as described above, current host-parasite associations are likely to be confounded by ongoing evolutionary processes, which may complicate the effective design of control interventions. Furthermore, it must be acknowledged that even if 'true multi-host parasites' are a fallacy, multiple host species may still affect the transmission dynamics and persistence of a parasite in its natural environment through a number of potential mechanisms. Direct interactions between different host species (e.g. through competition or predation) may have consequences for host-parasite contact rate, and therefore parasite persistence (i.e. 'encounter reduction' in Keesing *et al*., 2006). In addition, host species that are not susceptible to infection with a particular parasite species or strain (i.e. a non-competent host) may still "amplify" infection risk if they contribute to the size of a shared vector population, or "dilute" infection risk if they are infested by generalist vectors which are then unable to proceed to infect a competent host (Norman *et al*., 1999; Gilbert *et al*., 2001; Laurenson *et al*., 2003; LoGiudice *et al*.,

2003; Keesing *et al*., 2010; Randolph & Dobson, 2012; Wood & Lafferty, 2013). Identifying the general importance of such indirect effects of multi-host community composition and host dynamics on parasite infection risk and persistence, along with integrating parasite evolutionary dynamics into the design of control interventions, are therefore key research themes for the future of multi-host parasite ecology.

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